THE

JOURNAL OF EXPERIMENTAL MEDICINE

THE JOURNAL OF EXPERIMENTAL MEDICINE

EDITED BY

SIMON FLEXNER, M.D.

AND

EUGENE L. OPIE, M.D.

PUBLISHED FOR

THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH
NEW YORK

VOLUME TWELFTH

WITH SIXTY-NINE PLATES AND FIFTY-FOUR FIGURES IN THE TEXT

THE NEW ERA PRINTING COMPANY
1910

PRESS OF
THE NEW ERA PRINTING COMPANY
LANCASTER, PA.

CONTENTS.

No. 1, January 1, 1910.

PAGE.

I. Winternitz, M. C., and Pratt, J. P. On the Relation of the Catalytic Activity of the Blood to the Number of Red Blood Cells in Health, and to the Number of White Blood Cells and the Body Temperature in Peritonitis 1

II. Winternitz, M. C., and Rogers, W. B. The Catalytic Activity of the Developing Hen's Egg 12

III. Darling, Samuel T. Experimental Sarcosporidiosis in the Guinea-pig and its Relation to a Case of Sarcosporidiosis in Man 19

Plates I and II.

IV. Woglom, William H. Resistance Produced in Mice against Transplanted Cancer by Autoinoculation of the Spleen 29

V. Meltzer, S. J., and Auer, John. The Respiratory Changes of Pressure at the Various Levels of the Posterior Mediastinum 34

Plate III.

VI. Cooke, Jean V. The Excretion of Calcium and Magnesium after Parathyroidectomy 45

VII. Stewart, Hugh A. The Dextrose Consumption by the Isolated Perfused Human Heart 59

VIII. Meakins, J. C. An Experimental Study of Opsonic Immunity to Staphylococcus aureus 67

IX. Lewis, Paul A. Tuberculous Cervical Adenitis: A Study of the Tubercle Bacilli Cultivated from Fifteen Consecutive Cases 82

X. Ringer, A. I. The Influence of Adrenalin in Phlorhizin Diabetes 105

232875

No. 2, March 14, 1910.

XI. Winternitz, M. C., and Pratt, J. P. The Influence of Various Organs on the Catalytic Activity of the Blood 115

XII. Pearce, Richard M. Concerning the Depressor Substance of Dog's Urine and its Disappearance in Certain Forms of Experimental Acute Nephritis 128

Plate IV.

XIII. Carrel, Alexis. Peritoneal Patching of the Aorta 139

Plate V.

XIV. Carrel, Alexis. Remote Results of the Replantation of the Kidney and the Spleen 146

XV. Auer, John, and Lewis, Paul A. The Physiology of the Immediate Reaction of Anaphylaxis in the Guinea-pig 151

Plates VI to IX.

XVI. Terry, B. T. An Attenuated Surra of Mauritius with Immunity Tests after Recovery 176

XVII. Noguchi, Hideyo. Pleomorphism and Pleobiosis of Bacillus bifidus communis 182

Plates X to XIII.

XVIII. Pearce, Richard M., Hill, Miner C., and Eisenbrey, Arthur B. Experimental Acute Nephritis: The Vascular Reactions and the Elimination of Nitrogen 196

Plates XIV to XVII.

XIX. Flexner, Simon, and Lewis, Paul A. Experimental Epidemic Poliomyelitis in Monkeys ... 227

Plates XVIII and XIX.

No. 3, May 1, 1910.

XX. Eyster, J. A. E. Studies on the Venous Pulse. I. A Study of the Diastolic Waves of the Venous Pulse, with Especial Reference to the Possibility of a Wave Due to the Contraction of the Venous

Region of the Mammalian Heart 257
Plates XX and XXI.

XXI. GUTHRIE, C. C. Survival of Engrafted Tissues. I. (A) Ovaries and (B) Testicles 269
Plates XXII and XXIII.

XXII. BURTON-OPITZ, R., and WOLF, H. F. The Changes in the Venous Bloodflow on Administration of Amyl Nitrite 278
Plate XXIV.

XXIII. NASMITH, GEORGE G., and HARRISON, F. C. Changes Induced in the Blood of Rabbits by Living in an Atmosphere of Water Gas 282

XXIV. FLEISHER, MOYER S., and LOEB, LEO. Studies in Edema. VI. The Influence of Adrenalin on Absorption from the Peritoneal Cavity, with Some Remarks on the Influence of Calcium Chloride on Absorption..... 288

XXV. MARINE, DAVID, and LENHART, C. H. Observations and Experiments on the So-called Thyroid Carcinoma of Brook Trout (*Salvelinus fontinalis*) and its Relation to Ordinary Goitre 311
Plates XXV and XXVI.

XXVI. BERRY, JANE L., and MANN, A. The Complement Binding Test in Rabies 338

XXVII. ROUS, PEYTON. An Experimental Comparison of Transplanted Tumor and a Transplanted Normal Tissue Capable of Growth 344
Plates XXVII to XXX.

XXVIII. OPIE, EUGENE L. On the Relation of Combined Intoxication and Bacterial Infection to Necrosis of the Liver, Acute Yellow Atrophy and Cirrhosis 367
Plates XXXI to XXXVIII.

XXIX. FOWLER, C. C., and HAWK, P. B. Studies on Water Drinking. II. The Metabolic Influence of Copious Water Drinking with Meals 388

XXX. GIBSON, ROBERT BANKS, and BANZHAF, EDWIN J.

The Quantitative Changes in the Proteins in the Blood Plasma of Horses in the Course of Immunization 411

No. 4, JULY 23, 1910.

XXXI. AMBERG, SAMUEL. The Cutaneous Trichophytin Reaction 435

Plate XXXIX.

XXXII. CARREL, ALEXIS. Latent Life of Arteries 460

Plates XL to XLIII.

XXXIII. FLEISHER, MOYER S., and LOEB, LEO. Studies in Edema. VII. The Influence of Nephrectomy and Other Surgical Operations and of the Lesions Produced by Uranium Nitrate upon Absorption from the Peritoneal Cavity 487

XXXIV. FLEISHER, MOYER S., and LOEB, LEO. Studies in Edema. VIII. The Influence of Caffeine on Absorption from the Peritoneal Cavity and the Influence of Diuresis on Edema 510

XXXV. BROWN, W. H. Observations on the Origin, Distribution and Significance of Fuchsin Bodies, with Special Staining Technique 533

XXXVI. FIELD, CYRUS W. A Study of an Extremely Pure Preparation of Ricin 551

XXXVII. WRIGHT, JAMES HOMER. Neurocytoma or Neuroblastoma, a Kind of Tumor not Generally Recognized 556

Plates XLIV to XLVI.

XXXVIII. KNOX, J. H. MASON, MOSS, W. L., and BROWN, G. L. Subcutaneous Reaction of Rabbits to Horse Serum 562

No. 5, SEPTEMBER 1, 1910.

XXXIX. BOND, G. S. Effect of Various Agents on the Blood Flow through the Coronary Arteries and Veins 575

Plates XLVII to LI.

XL. Hirschfelder, Arthur D. Another Point of Resemblance between Anaphylactic Intoxication and Poisoning with Witte's Pepton 586
Plate LII.
XLI. Levin, Isaac. Studies on Immunity in Cancers of the White Rat 594
XLII. Wells, H. Gideon. The Relation of Fatty Degeneration to the Oxidation of Purines by Liver Cells 607
XLIII. Nichols, Henry J. Experimental Yaws in the Monkey and Rabbit 616
Plates LIII and LIV.
XLIV. Brown, W. H. Changes in the Hemosiderin Content of the Rabbit's Liver during Autolysis 623
XLV. Auer, John. The Effect of Vagus Section upon Anaphylaxis in Guinea-pigs 638
Plates LV and LVI.
XLVI. Duval, Charles W. The Cultivation of the Leprosy Bacillus and the Experimental Production of Leprosy in the Japanese Dancing Mouse. 649
Plates LVII to LX.
XLVII. Dochez, A. R. Intracellular Proteolytic Enzymes of Liver 666
XLVIII. Sachs, B., and Strauss, I. The Cell Changes in Amaurotic Family Idiocy 685
Plates LXI to LXV.
XLIX. Rous, Peyton. A Transmissible Avian Neoplasm. (Sarcoma of the Common Fowl) 696
Plates LXVI to LXVIII.

No. 6, November 1, 1910.

L. Klotz, Oskar. Concerning Compensatory Hyperplasia of the Intima 707
Plate LXIX.
LI. Craig, Charles F. Observations upon the Noguchi Modification of the Wassermann Comple-

ment Fixation Test in the Diagnosis of Lues in the Military Service 726
LII. McCrae, John, and Klotz, Oskar. The Distribution of Fat in the Liver 746
LIII. Winternitz, M. C., and Rogers, W. B. Observations upon the Action of Salts on the Catalase of the Blood in Rabbits 755

ON THE RELATION OF THE CATALYTIC ACTIVITY OF THE BLOOD TO THE NUMBER OF RED BLOOD CELLS IN HEALTH, AND TO THE NUMBER OF WHITE BLOOD CELLS AND THE BODY TEMPERATURE IN PERITONITIS.[1]

By M. C. WINTERNITZ and J. P. PRATT.

(*From the Pathological Laboratory of the Johns Hopkins University.*)

Before entering into the discussion of the main object of this paper, namely, the relation of the catalytic activity of the blood to the white blood cells and the body temperature in peritonitis, a heretofore obscure finding might be explained to a certain degree, at least.

It will be remembered[2] that the catalytic activity of the blood of a normal rabbit is practically constant from day to day, while the activity of the blood of different rabbits may vary within relatively wide limits. This phenomenon seemed unexplainable except for the fact that in rabbits of the same litter and the same grade of development there was a greater correspondence of the activity of the blood than in those where there was a visible difference in size and age. The relation of the catalytic activity of the blood to the number of red blood cells may, as will be seen, explain these facts.

THE RELATION OF THE CATALYTIC ACTIVITY OF THE BLOOD TO THE RED BLOOD CELL COUNT.

Bergengrün was able to show as early as 1888 that the power of the blood to decompose hydrogen peroxide depended almost entirely upon the red blood cells, and while the white blood cells and the blood serum are also capable of splitting hydrogen peroxide, their action is so slight that it is hardly to be taken into consideration when compared to the great activity which the red blood cells exhibit. This work has been repeatedly confirmed, and, needless to say, if

[1] Received for publication August 5, 1909.

[2] Winternitz, *Jour. of Exper. Med.*, 1909, xi, 200.

any practical value is to come from the determination of the catalytic activity of the blood, its relation to the number of red blood cells, which apparently are the source of the blood's action, must be known both in health and in disease.

We have selected a number of healthy rabbits and carefully determined their red blood count and the power of their blood to decompose hydrogen peroxide. In order that the counts should be of greater value, we removed five cubic centimeters of blood from the ear vein of each rabbit and placed this in a graduated centrifuge tube with ten cubic centimeters of sodium citrate solution. The tubes were then centrifuged for a definite length of time and the volume of the red blood cells noted. In the table given below, we have recorded for comparison the volume of red blood cells, the red blood count and the catalytic action of the blood in each case. The table represents three series of experiments, and in calculating the relation of volume to number of red blood cells, it should be remembered that only the members of the same series are comparable, as they represent the corpuscles which were centrifuged for the same length of time.

No.	Red blood count.	Vol. of R. B. C. in 5 c.c. of blood.	Catalytic activity of blood (1-200 dilution.) Seconds.							
			15	30	45	60	75	90	105	120
1	5,160,000	1.8 c.c.	20.6	26	29.8	33	35.6	38.2	40.4	42.2
2	4,870,000	1.7	20.8	26.4	30	33.2	35.2	39	41.8	43.6
3	6,292,000	2.2	32.6	39.8	44.6	47.6	50.2	57.4	52	52.4
4	4,590,000	1.62	20.4	25.8	28.8	32	35.2	37.4	40.4	43
5	4,192,000	1.45	20.4	25.2	29	32.8	35.6	39.2	42.4	45
6	5,320,000	1.95	24	31.4	37	42	46	49	51.6	53.2
				Second series.						
1	4,488,000	1.7 c.c.	17.6	22.4	25.6	28	30.8	33.2		
2	6,118,000	2.4	20	24.4	28	31	34	36.4		
3	4,000,000	1.5	18.8	22.8	26.6	29.8	32.6	35.4		
4	5,416,000	2.15	19.8	24.2	28.6	32	35.4	38		
				Third series.						
1	4,760,000	1.82 c.c.	24.6	31.6	37	42	45.4	47.6		
2	5,314,000	2.00	24.6	31.4	37	41.8	45.2	47.2		
3	4,364,000	1.65	19.4	24.8	29.6	33.6	37	39.6		
4	2,356,000	0.96	14.4	17.8	20.8	23.8	26.4	28.4		

It will be seen at a glance from the above table that there is, as has been frequently shown, a definite relation between the volume and number of red blood cells. On the other hand, while there is

a definite relation between the catalase and the red blood cells, these do not correspond exactly; for example, in Series 2, with a high blood count of over six millions, one would expect the catalytic activity to be very great, which, however, is not the case. It is

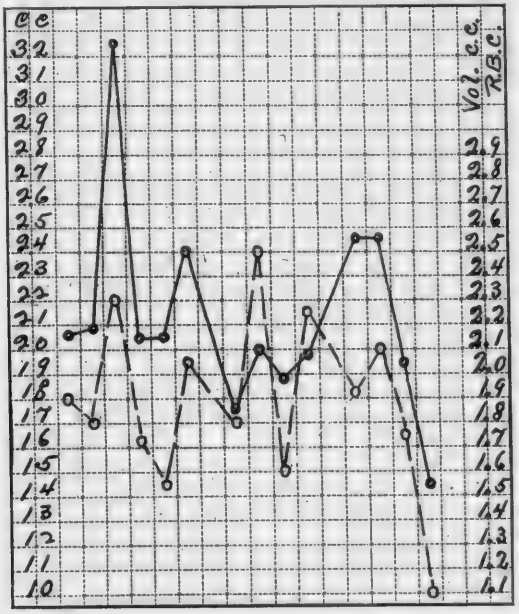


CHART 1. Showing relation of volume of red blood corpuscles in 5 cubic centimeters of blood to catalytic action of the blood.

only fair to say, then, that in most cases a very high blood count is accompanied by a high catalytic action and vice versa; lesser differences in the blood count do not appear to be of equal importance in varying the intensity of the action of the blood towards hydrogen peroxide.

We have attempted to arrive at the relation of the red blood count and the catalytic activity of the blood by still another method. After determining both of these factors for a healthy rabbit, the animal was bled four times in relatively rapid succession and the blood count and the catalytic activity determined after each bleeding.

The first five cubic centimeters of each bleeding were caught in sodium citrate solution and later centrifuged in graduated tubes to determine the volume of corpuscles. The results are expressed in the following table:

Catalase.

Time.	Number of R. B. C.	Vol. in c.c.	Seconds. 15	30	45	60
10.30 a.m.	3,400,000		17	21.2	25.2	28.4
10.45 a.m.	2,640,000	1.15	16	20	23.2	26.4
11.30 a.m.	1,888,000	1	14.8	18.6	21.4	23.8
11.45 a.m.	1,712,000	0.8	13	16.6	18.6	21.2
12.30 p.m.	1,416,000	0.6	11	14	16.4	18.6
12.45 p.m.	1,392,000		9.2	12	13	15.8

After the volume of corpuscles had been determined, half a cubic centimeter of corpuscles was removed from each tube and a 1 to 800 dilution made in distilled water. Five cubic centimeters of these solutions were then determined for their catalytic power and, as will be seen from the table below, the activity of the corpuscles removed was the same in each case.

Red Blood Corpuscles in Dilution 1–800.

Bleeding.	Vol. of R. B. C.	Seconds. 15	30	45	60
1	1.15	42	50	50.8	51.2
2	1.0	41.6	50	50.4	50.6
3	0.8	41	49.4	50.6	50.8
4	0.6	41.2	49.8	50.2	50.6

The intimate relation between the catalytic activity of the blood and the number and volume of red blood cells is clearly emphasized in the above experiments. While the number of red blood cells varies directly with the catalytic action of the blood in different animals, the variation is not always proportional, and so in the same animal the curve of the catalytic activity will depend greatly on the number of corpuscles, even though the decrease in the power of the blood to split hydrogen peroxide may not be as great as the decrease in the number of red blood cells after bleeding.

The problem, therefore, which confronted us in the beginning of this work, namely, the variations found in the catalytic activity of the blood of healthy rabbits, seems somewhat clearer. Large healthy animals are likely to have a high red blood content, and also

a high catalytic activity. Animals of the same litter are likely to be of the same general state of development, and, therefore, having about the same red blood count will probably have about the same power of decomposing hydrogen peroxide in their blood.

It still remains for us to determine the relation of the red blood count to the variations found in the catalytic activity of the blood in disease, and experiments with this point in view are now being carried on.

In a previous article,[3] it was shown that as a result of experimentally produced peritonitis in rabbits, whether the latter was brought about by bacteria or as a result of extravasation of irritant substances like urine, there was a rise in the catalytic activity of the circulating blood as determined by the method employed. It was shown that this rise was definitely determinable within two hours after the bacteria had been introduced into the peritoneal cavity, and that the catalytic activity of the blood steadily increased, reaching its maximum in some cases within six hours from the beginning of the experiment, while in others it remained excessively high for more than thirty-six hours from the time the peritonitis had commenced.

It occurred to us at that time that the determination of the catalytic activity of the blood might be of value clinically in determining the onset of serous cavity infections, and especially the peritonitis in cases of typhoid fever, appendix rupture, etc. Work is now being done with this point in view in the wards of the Johns Hopkins Hospital and will be reported in detail later.

In the course of our work this year, we have had numerous opportunities of confirming the results previously obtained on animals. We have studied many cases of infected rabbits with this point in view, but more interesting possibly than these are several cases in which as a result of some operative procedure where an entirely different result was sought, a peritonitis ensued. It may be of interest to cite one of these cases in detail.

Rabbit No. 57.—Splenectony. The operation was apparently satisfactory, and the animal recovered promptly. It was slightly drowsy on the second day, but this would have caused no particular anxiety if there had not been such a rapid and marked rise in the catalase of the blood. We have learned that this

[3] *Jour. of Exper. Med.*, 1909, xi, 120.

is not what is to be expected following splenectomy. This rise continued for several days, as shown by the chart, even though the animal did not seem particularly ill. Its general condition became worse and on April 23 a tumor, which had been present when the animal was admitted, was explored. The mass lay in the subcutaneous tissue of the left back, and on incision a large encapsulated mass of very foul smelling pus was revealed.

At autopsy the animal was much emaciated. The abdomen contained a small amount of blood-stained fluid. Over the peritoneal surfaces there was a slight friable exudate of grayish material which in places bled on removal. The exudate was most abundant in the upper portion of the abdomen just beneath the operative incision. Several of the sutures had sloughed, and here the bowel was glued to the parietal peritoneum. Microscopically, the exudate was composed of fibrin and leucocytes; the latter, however, were poorly preserved. This exudate showed everywhere an invasion by capillaries about which numerous young fibroblasts and round cells could be seen.

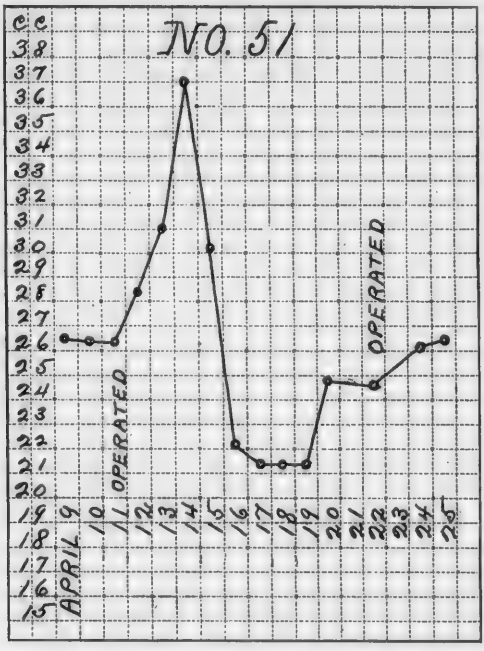


CHART 2. Showing curve of activity during first fifteen seconds.

It will be seen from Chart 2 that with the peritonitis there was a rise in the catalytic activity of the blood, and this was the first indication that the animal was ill, since clinically it showed practically

no untoward signs. This case suffices to emphasize the change in the catalytic activity of the blood following peritonitis, and furthermore shows that it may be the first sign of such infection in animals.

The work to be reported was undertaken with the hope of determining the relation of the catalytic activity of the blood to the body temperature and to the number of white blood cells.

THE RELATION OF THE CATALYTIC ACTIVITY OF THE BLOOD TO THE BODY TEMPERATURE AND THE NUMBER OF WHITE BLOOD CELLS.

As is well known, the body temperature of normal rabbits varies within wide limits, the normal ranging between 98° F. and 102° F.

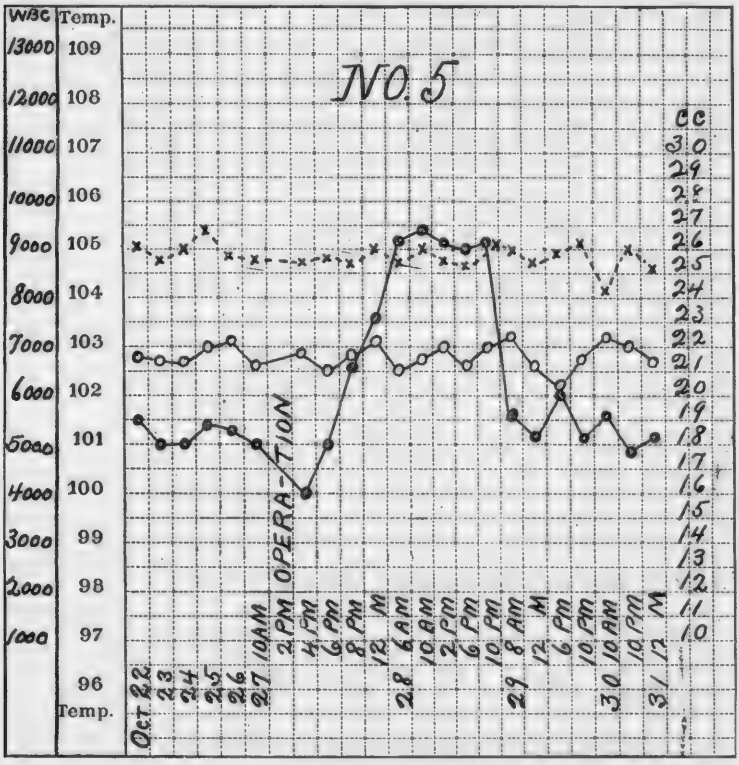


CHART 3.

This variation has no effect upon the catalytic activity of the blood, for it has been previously shown that the catalase of a single animal's blood is constant from day to day,

In the first series of experiments concerning the relation of the body temperature and number of white blood cells to the catalytic

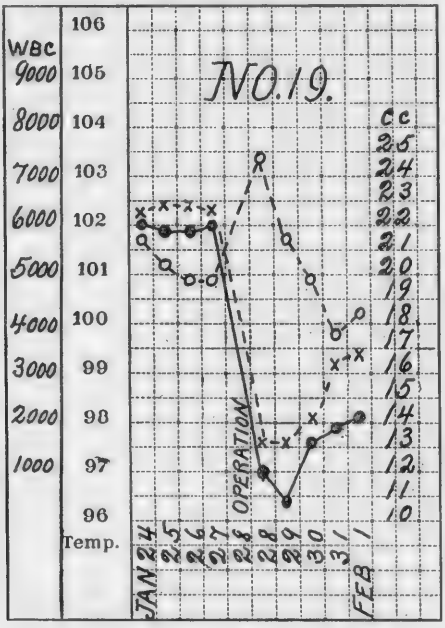


CHART 4.

activity of the blood, it was attempted to produce a hyperpyrexia alone. This was accomplished by puncturing the heat regulating center described by Aronsohn and Sachs[4] which they located in the anterior medial portion of the corpus striatum. We are indebted to Dr. W. G. MacCallum who kindly performed this operation for us in several cases.

Chart 3 represents the catalytic activity of the blood as determined in the first fifteen seconds (hollow dots) and its relation to the body temperature (solid dots) and white blood cells (crosses).

[4] *Arch. f. d. ges. Physiol.*, 1885, xxxvii, 232.

Chart 3 illustrates clearly that while there may be a marked hyperpyrexia resulting from a puncture of the anterior portion of the corpus striatum there is no corresponding change in the catalytic activity of the blood or the leucocyte count.

The two following experiments illustrate further the entire inde-

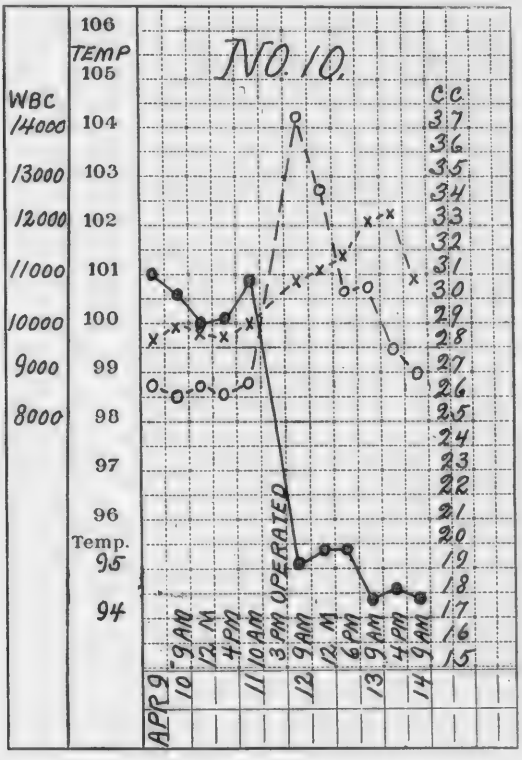


CHART 5.

pendence of the body temperature, the catalytic activity of the blood, and the leucocyte count.

Rabbit No. 19.—In this case, a peritonitis was caused by the introduction of potato shavings saturated with a culture of *Staphylococcus aureus.* The animal lived four days. At autopsy the abdominal cavity contained a considerable amount of fibrino-purulent exudate, the fibrinous portion being most abundant about the potato shavings. Throughout the viscera there were many miliary abscesses to be made out.

Chart 4 illustrates that even though there may be a decided fall in the body temperature and the leucocyte count, the catalytic activity of the blood may rise above normal.

Rabbit No. 10.—Here the peritonitis was caused by ligating the distal end of one ureter and slitting this proximal to the ligature so that the urine would drain into the abdominal cavity. The animal lived for forty-three hours, when it was sacrificed. The autopsy showed an extensive fibrino-serous peritonitis.

In this case (see Chart 5) there is a rise in the leucocytes together with the rise in the catalase of the blood, while the body temperature shows a decided fall.

Rabbit No. 15.—In this case a small portion of aleuronat was suspended in normal saline solution, and injected into the left pleural cavity. The animal

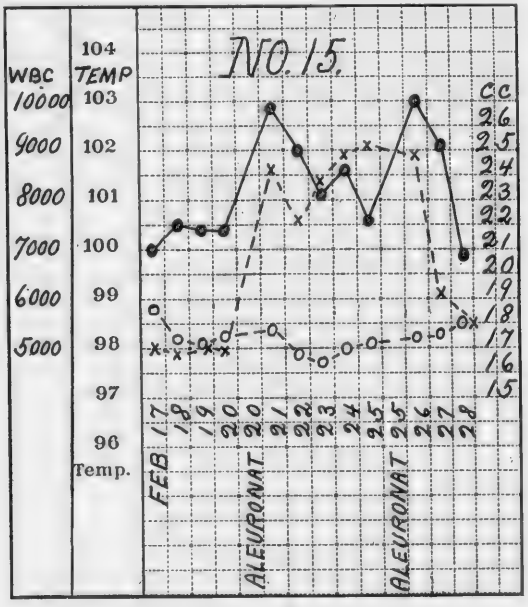


Chart 6.

was only slightly ill the next day and rapidly recovered as far as could be judged from external appearances. Several days later he received a second injection which proved fatal.

In Chart 6 there is no change in the catalytic activity of the

blood but a rise in both the body temperature and the white blood cells.

CONCLUSIONS.

The catalytic activity of the blood of normal rabbits varies almost directly with the volume and number of red blood cells. This explains to a certain extent at least why animals of the same general degree of nutrition, and of the same litter, should have about the same activity since they are likely to have the same number of red blood cells, and why healthy large animals should read high while small poorly nourished ones should read low.

Accompanying the hyperpyrexia resulting from puncture of the corpus striatum of a rabbit's brain, there is no change in either the catalytic activity of the blood or the white blood count. In experimentally produced peritonitis, the catalytic activity of the blood always rises, and is, therefore, absolutely independent of body temperature and white blood cells since one or both of these may rise, fall or remain stationary while the catalytic action increases.

THE CATALYTIC ACTIVITY OF THE DEVELOPING HEN'S EGG.[1]

By M. C. WINTERNITZ and W. B. ROGERS.

(*From the Pathological Laboratory of the Johns Hopkins University.*)

Battelli and Stern (1) have studied the catalytic activity of the tissues of embryo guinea-pigs and of newly born pigs. They showed that some tissues, among them the spleen and the blood, possess even at birth a power of decomposing hydrogen peroxide equal to that of the adult pig. The kidney, on the contrary, and above all the liver, are much less active in this respect in newly born animals, but rapidly increase in activity until at the sixth or seventh day the catalytic activity of these organs has reached that of the adult. The muscle and the brain are also less active at birth than in adult life, but the difference is not great. They conclude that the amount of catalase in a single organ has a certain relation to the intensity with which that organ functions.

Mendel and Leavenworth (2) likewise have studied the various tissues of embryo and adult pigs. Like Battelli and Stern, they find adult liver and kidney most active. One is unable to decide between these two organs, since practically the maximal activity attainable with the amount of hydrogen peroxide they used is reached in the first fifteen seconds of their experiment. The adult lung, muscle and brain have much less activity in the order named, according to their results. They find that the tissues of embryos sixty-five millimeters long have abundant power to decompose hydrogen peroxide.

We have found the following relation between the catalytic activity of various organs of newly born and adult rabbits.

It will be readily seen from the above table that there is very little difference in the catalytic activity of the tissues of adult and newly born rabbits. The difference in the activity of the blood is no

[1] Received for publication August 5, 1909.

TABLE I.

Adult Rabbit.

	Seconds.					
	15	30	45	60	75	90
Blood (1–200 dilution)	22	27.8	34.2	38.4	41.6	43.8
Kidney (1–40 dilution)	48	52.2	52.2			52.2
Liver (1–40 dilution)	32.5	44	48	50.4	51.2	51.6
Lung (1–40 dilution)	31.4	42	47	50	51	51.2
Spleen (1–40 dilution)	25.6	37	45	49	50.6	50.8
Brain (1–40 dilution)	3.4	4.4	5.2	5.8	6.6	7.2
Muscle (1–40 dilution)	2.4	3.2	3.8	4.4	4.9	5.4
Rabbit 27 Hours Old.						
Blood (1–200 dilution)	17	22	26.6	30.6	34	37
Liver (1–200 dilution)	50	51.8				51.8
Kidney (1–200 dilution)	23	32	38.6	44	47.6	49.4
Lung (1–200 dilution)	22.2	29.2	35	40	44	48
Brain (1–200 dilution)	3.2	4.4	5.6	6.2	6.8	7.4
Muscle (1–200 dilution)	2.4	3.6	4.2	4.8	5.4	6.4
Rabbit 5 Days Old.						
Blood (1–200 dilution)	17	23.4	28.2	31.6	35.4	38.4
Liver (1–40 dilution)	53					53.2
Kidney (1–40 dilution)	30	43	48.5	51.3	52.4	
Lung (1–40 dilution)	22	30	36.6	42	46.4	49
Brain (1–40 dilution)	3.8	4.8	5.6	6.2	6.8	7.6
Muscle (1–40 dilution)	2.6	3.4	4	4.6	5.4	6.2

greater than is often found between adult animals which are apparently quite healthy. This has been remarked upon in an earlier paper by one of us (3).

Otherwise, the livers of rabbits twenty-seven hours and five days old seem more active than that of the adult, while the activity of the kidneys and lungs seems to be somewhat increased in the latter.

It occurred to us that it might be of interest to study the catalase of single ova which were isolated from the body and which after fertilization developed entirely independently. In this way the contents of the ovum could be readily determined and if there was any increase in its activity with the development of the embryo, the conclusion must necessarily be that the catalytic activity developed with the cell growth from the constituents of the egg. The hen's egg is peculiarly adapted to this experiment and it was studied with the following points in view:

1. What is the catalytic activity of the entire incubated egg, of the white, of the yolk, and of the germinal center?

2. What changes occur in the catalytic activity of the unfertilized incubated egg?

3. Is there an increase in the catalytic activity of the incubated fertilized egg, and if so, does it increase in all of the parts of the egg or only in the germinal center?

4. Does the increase in the catalytic activity of the developing egg correspond grossly to the development of any single part of the embryo, as the vascular system, etc.?

Two series of eggs procured fresh were used. A part of each series was tested immediately for catalytic activity, a second portion was left unincubated for varying periods up to two and one-half months, the third portion was incubated at approximately 37° C., and tested for activity at intervals of twenty-four hours.

The results obtained from the different procedures with the two series were practically the same. The amount of catalase present was determined according to the amount of oxygen liberated by the action of the egg, or part of the egg, on a definite amount (five cubic centimeters) of neutralized hydrogen peroxide, readings being made at intervals of fifteen seconds, according to the method described by Kastle and Loevenhart (4).

TABLE II.

Fresh,[2] *Whole, Unincubated Eggs.*

Seconds	15	30	45	60	75	90	105	120
	1.6	3.6	4.9	6	6.9	7.7		8.9
	0	1	4	6	9	11		
	1	2.2	4.5	7	8.8	10.4	11.8	13

It will be readily seen that the catalytic activity of a fresh unincubated hen's egg is very low and constant for different eggs. There is, however, a definite decomposition of the hydrogen peroxide, in one case as much as thirteen cubic centimeters of oxygen being evolved within the first two minutes.

TABLE III.

Unincubated Egg, Kept from 2½ to 3 Months.

Seconds.	15	30	45	60	75	90	105	120
	1	3	4.5	5.2				

[2] The eggs were obtained fresh from the country within three days after laying and tested immediately.

Here the results are practically identical to those obtained in Table II, despite the fact that the eggs have been kept without any precaution against bacterial invasion.

TABLE IV.

Germinal Portion of Unincubated Eggs.

Seconds.	15	30	45	60	75	90	105	120
	1	1.2	1.4	1.6	1.6	1.8	1.9	2
	1.6	1.9	2.2	2.3	2.4	2.6		
	1.1	1.4	1.5	1.6	1.8	1.9	2	2

There was so little decomposition of the hydrogen dioxide when only the germinal portion of the unincubated egg was used that it occurred to us that this might really be brought about by the mechanical agitation. That this is true can be readily determined by referring to Table VII, where only hydrogen peroxide and sterile water were shaken together. The conclusion we naturally draw is that the cells of the germinal center of unincubated eggs have probably no catalytic power.

TABLE V.

White of Unincubated Egg.

Seconds.	15	30	45	60	75	90	105	120
	0	0.6	0.8	1.3	1.6	1.8	2	2.2

TABLE VI.

Yolk of Unincubated Egg.

Seconds.	15	30	45	60	75	90	105	120
	0	0.6	1	1.2	1.4	1.5	1.7	1.9

TABLE VII.

Water (5 c.c.) Alone, Shaken as Control.

Seconds.	15	30	45	60	75	90	105	120
	1.4	1.7	2	2.1	2.2	2.4	2.5	2.7

It is interesting to note that when the egg is separated in the above manner and each part tested separately concerning its power to split hydrogen peroxide there should be no marked activity in any part, and, moreover, that the sum of their actions is much less than when they are all tested together.

TABLE VIII.

Whole Unfertilized Egg Incubated (No Development).

Time in days.	Seconds. 15	30	45	60	75	90	105	120
15	1.2	1.6	2	2.2				
21	0.6	1	1.4	1.6	1.8	2	2.2	2.3

The above table needs little comment. Just as the egg does not vary in its catalytic activity at room temperature over long periods of time, so there is no increase in the activity of undeveloped incubated eggs.

TABLE IX.

Germinal Portion of Incubated Eggs.

Time in days.	Seconds. 15	30	45	60	75	90	105	120
1	1	1.1	1.3	1.4	1.5	1.6	1.6	1.6
2	1.8	2.2	2.8	3	3.2	3.4	3.6	3.8
3	2	2.7	3.2	3.5	3.8	4	4.2	4.5
4	2.2	4.2	5.4	6	6.4	6.6	7	7.4
5	8	14.5	20	24	28	31	33.5	36
6	4.5	9	11.5	13.5	15	17	19	20
7	11	20	28	33	37.5	41	44	46
8	13.2	24.2	33	38.6	43.8	46.8	49.4	51
9	16.5	28.5	36	41.2	45.2	47.8	49.7	51.1
15	35	47	49	49.6	50	50		

The above table and curve illustrate clearly the rapid and progressive rise in the catalytic power of the developing germinal center.

TABLE X.

Yolk and White of Incubated Eggs.

(Series I.)

Time in days.	Seconds. 15	30	45	60	75	90	105	120
2	0	0	0	0				
3	1.8	2.4	3	3.6				
4	1.8	2.4	3	3.6	4	4.5	5	5.3

(Series II.)

1	1.4	2.4	4	5.4	6.5	7.4	8.2	9
2	0.6	1	1.4	1.8				
	0.6	0.8	3.6	4.4	5	5.4	6.2	6.8
3	0.6	1	1.8	2.2	2.7	3.2		
4	0	0.4	0.8	1.4				
5	0.1	0.4	1	1.4				
9	0.8	1.2	1.5	1.8				

TABLE XI.

Yolk of Incubated Eggs.

Time in days.	Seconds. 15	30	45	60	75	90	105	120
4	0.4	1.0	1.6	2	2.6	3	3.3	3.6
5	0.6	1	1.6	2	2.6			
6	0.7	1.2	1.7	2.4				
7	0.4	0.8	1.2	1.8				

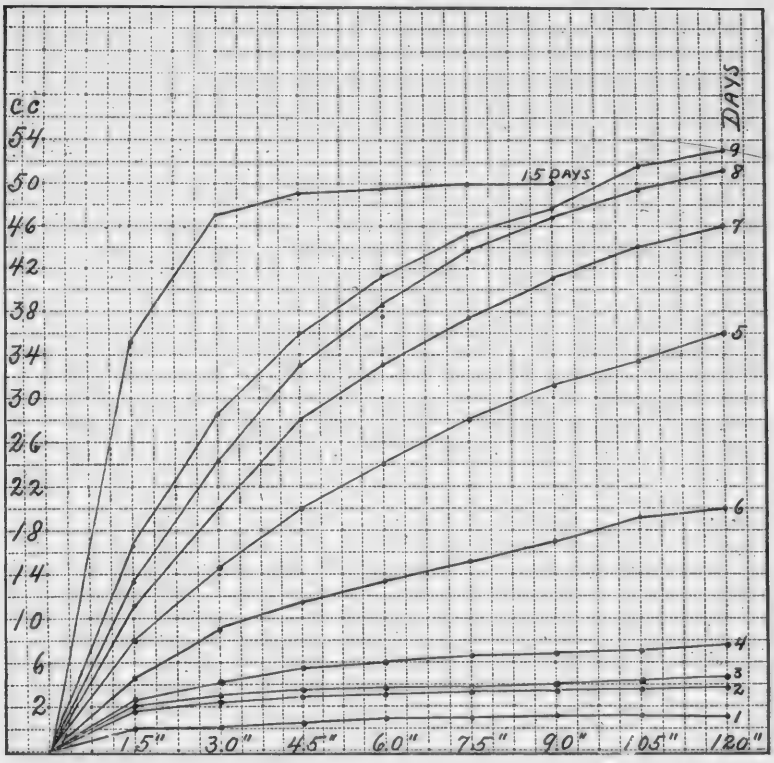


TABLE XII.

Amniotic Fluid of Incubated Eggs.

Time in days.	Seconds. 15	30	45	60	75	90	105	120
11	2.2	2.6	2.8	3				

Tables X and XI show that the yolk and white together or separately do not develop the power of splitting hydrogen peroxide, nor is this power to be found in the amniotic fluid.

It seems, therefore, that with cell development and the formation of the embryo there is also a development on the part of the germinal portion to decompose hydrogen peroxide catalytically. This power is not present to any similar extent in fresh eggs or in unfertilized incubated eggs, and it does not form in the parts of the egg other

than the germinal center. Since the ingredients of the egg are the same, excluded as it is from all outside influences but the air, it seems permissible to conclude that the ferment action towards hydrogen peroxide or catalase is formed within the egg itself by the developing cells from the constituents of the egg.

CONCLUSIONS.

A. *The catalytic activity of the tissues of newly born and adult rabbits.*

1. There is practically no difference between the catalytic power of the blood, brain or muscle of newly born and adult rabbits.

2. The kidneys and lungs are less active in the newly born, but increase rapidly during the first days of life.

3. The liver is more active in the newly born than in the adult rabbit.

B. *The catalytic activity of the developing hen's egg.*

1. The entire fresh egg has a slight but definite catalytic power which remains practically constant even though the egg be kept for several months at room temperature.

2. The separate parts of the egg, germinal center, yolk and white have practically no activity when tested individually.

3. The entire unfertilized egg shows no catalytic activity even after incubation at 38° C. for twenty-one days.

4. The germinal portion of the incubated fertilized egg rapidly acquires the power of decomposing hydrogen peroxide, while the yolk and white together or separately show no such increase, nor is there any activity to be found in the amniotic fluid.

5. It seems, therefore, that the catalytic power of the developing egg is formed from the contents of the egg which themselves show little action, by the developing germinal portion, independent of all external influences except heat (38° C.) and air.

BIBLIOGRAPHY.

1. Battelli and Stern, *Arch. di Fisiol.*, 1905, ii, 471.
2. Mendel and Leavenworth, *American Jour. of Physiol.*, 1908, xxi, 69, 76, 77, 85, 95, 99.
3. Winternitz, *Jour. of Exper. Med.*, 1909, xi, 200.
4. Kastle and Loevenhart, *American Chem. Jour.*, 1903, xxix, 397, 563.

EXPERIMENTAL SARCOSPORIDIOSIS IN THE GUINEA-PIG AND ITS RELATION TO A CASE OF SARCOSPORIDIOSIS IN MAN.[1]

By SAMUEL T. DARLING, M.D.

Ancon, Canal Zone.

Plates I and II.

Notwithstanding the fact that infections by representatives of the genus *Sarcocystis* are very common among some of the domestic animals, little is known about these interesting sporozoa, and very few experiments have been conducted for the purpose of clearing away our ignorance of the mode of infection and the life history of the parasites in their various hosts. There are at least two reasons for this: first, sarcosporidiosis gives rise to few, if any, symptoms; and secondly, the infection is very rarely communicated to man.

Sarcosporidia have been found in the tissues of mammals, birds and reptiles, by different observers in various parts of the world, more than twenty-eight species of hosts being represented. Some of the domestic animals are infected to a very high degree; 98 per cent. of sheep in Europe are said to be infected with sarcosporidia, and 98.5 per cent. of swine. Theobald Smith (1) found that 6 2/3 per cent. of the gray mice in his laboratory at Boston were naturally infected. The writer (2), from an examination of rats caught in or near the City of Panama in 1908, found the following number of naturally infected animals:

		Number infected to gross examination.	Per cent. infected to gross examination.
Number of adult rats examined.....	131	15	11.4
Number of immature rats examined...	89	0	0
Number of adult rats, *Mus rattus*, examined	28	2	7.1
Number of adult rats, *Mus decumanus*, examined	65	7	10.7

[1] Received for publication September 7, 1909.

Number of rats examined, both species, all ages	324	22	6.7
		Number of these found to be infected upon microscopic examination.	Per cent. infected upon microscopic examination.
Number of rats examined that were not infected to gross examination	26	2	7.6

From this table it appears that adult rats alone show gross evidences of infection, and that some of the rats negative to gross examination may be found to be infected upon microscopic examination; that is, the sarcosporidia are young, not fully developed, and are obscured by being enclosed within a muscle fiber (Fig. 1). The larger proportion of infected specimens of *Mus decumanus* is in keeping with our knowledge of the habits of this species. The following is an illustration of this:

On October 20, 1908, 15 gray rats (*Mus decumanus*), and 15 black rats (*Mus rattus*) were placed in a roomy, well ventilated cage about 3 by 4 by 4 feet, to be fed with the carcasses of rats infected with *Sarcocystis muris*. Within a few days all the black rats had been killed and eaten by the gray rats, although they had been regularly supplied with food (bread and killed infected rats). It is not uncommon, also, to see gray rats kill and eat individuals of their own species.

Sarcosporidiosis in man, as Theobald Smith (1) has pointed out, has been rarely observed. This is because the infection is extraordinarily rare and also, no doubt, for the reason that the "muscular system in man is not subjected to that scrutiny which the viscera undergo in pathological enquiries, and that sarcosporidia may be present and yet not be recognized."

There are three assured instances of this infection in man. Kartulis (3), who reported the first case in 1893, found Miescher's cylinders of various sizes in the liver (?) and muscular system of a Sudanese who had succumbed to multiple abscesses of the liver and abdominal muscles. Döflein (4) regarded this case as doubtful. Braun (5), however, after examining Kartulis's preparations agreed with the latter's diagnosis of sarcosporidiosis.

The second case was that reported by Baraban and St. Remy (6) in 1894. These observers found sarcosporidia in the laryngeal muscles of a man who had been executed by hanging. The sporozoa distended the muscle fibers to four times their normal thickness,

and were 1.6 millimeters long and 0.17 millimeter wide, with a thin capsule. The sporozoites were banana-shaped, and were 8 to 9 microns long in sections. All individuals were in the same stage of development and the sporoblasts developed in room systems (Kammerung).

The third positive case was found by the writer (2) and was reported in April, 1909. The parasites were found in tissue taken from the right and left biceps (Figs. 2 and 3). They were microscopic in size and disappeared within a period of four months after the first examination. This case is of special interest because the parasite was discovered in the tissues of its living host, who was under observation for a period of one year, during which time tissue for examination was removed on three occasions. The close resemblance between the sarcosporidia in this case and those in guinea-pigs experimentally infected with *Sarcocystis muris* from rats was very striking.

In the writer's description of the case of sarcosporidiosis in man, he stated that the infection was probably a chance one by sarcosporidia whose customary habitat was some one of the domestic animals, or still lower order of life, and that the sporozoon described was probably abortive. This opinion has since become more firmly fixed by the results of Negri's (7) experiments, and by the results of the experiment described here in confirmation of Negri's in which a number of guinea-pigs were fed with the muscle of rats infected with *Sarcocystis muris.* In this experiment it was demonstrated that after an interval of five months from the last feeding the guinea-pigs had become infected, and parasites were found in sections of the thigh muscles. A most interesting and instructive fact developed from the experiment was that the sarcosporidium of the rat became profoundly modified in the muscles of the guinea-pig; that it became greatly reduced in size; that its mode of development in room systems was probably altered; and that, on the whole, it was strikingly like the sarcosporidium found in man (writer's case).

Theobald Smith (1), in a series of carefully conducted feeding experiments, demonstrated that mice could be infected by feeding them with infected muscle of mice.

Nègre (8) and Koch have also infected mice by feeding them

with infected muscle of other mice. Negri (7) has recently succeeded in infecting guinea-pigs with sarcosporidia by feeding them with muscle of rats infected by *Sarcocystis muris*. His experiments are of considerable importance in showing that guinea-pigs may become infected with *Sarcocystis muris* in this way, and also that the sarcosporidium undergoes an alteration in morphology in the muscle of the guinea-pig. This last observation, as Negri says, deserves consideration from a systematic standpoint, for inasmuch as the sarcosporidia attain special characteristics in the new host, they might be mistaken for a new species.

Negri (7) selected guinea-pigs in his experiments for the reason that these animals were not naturally infected so far as is known. His guinea-pigs were fed with infected rat's muscle, chopped and mixed thoroughly with bread or bran moistened with saline solution. The guinea-pigs were killed at intervals after the last feeding and sections of their pectoral muscles chiefly examined microscopically for sarcosporidia. Nine out of eleven animals fed became infected fifty days after the first feeding.

These sarcosporidia from guinea-pigs, described by Negri, so closely resembled those found by the writer in man that the following experiment was carried out to determine whether guinea-pigs could be infected in this tropical region, which is more nearly within the climatic limits of their natural habitat (Brazil) than in Pavia, Italy, where Negri's experiments were carried out, and also to determine whether there was any likeness between the guinea-pig sarcosporidia and those found in man (writer's case).

Six adult guinea-pigs, male and female, were placed in a clean pen October 19, 1908. The animals had been bred at the laboratory since 1905 from a lot obtained in the United States. They had always been fed with coarse fresh grass from the hill near by, which they received morning and evening. In this experiment the infected muscle was fed in the following way: on the morning of a feeding their regular supply of grass was withheld, and a much smaller amount was cut up into pieces, 1 to 3 cm. in length; this was placed in a zinc dish to prevent waste and was sprinkled over with a saline suspension of teased-out infected muscle or of teased-out ripe parasites from richly infected muscle.

October 19, 1908. Fed with teased chopped emulsified infected rat's muscle mixed with chopped grass blades and stalks.

October 26. Fed as on October 19.

October 31. On this date the method of feeding was changed by first teasing out the ripe sarcosporidia in saline solution from heavily infected rat's

muscle. The capsules of the sarcosporidia were torn so as to liberate the sporozoites and the suspension was then sprinkled over chopped grass blades and stalks.

November 2. Fed as on October 31.
November 17. Fed as on October 31.
November 27. Fed as on October 31.

The most favorable feeding was, probably, that of October 31, when a very large number of parasites were fed. The suspension, when examined microscopically, was found to contain innumerable adult mobile sporozoites. The guinea-pigs remained well and vigorous and, save for the first feeding, when only one guinea-pig was seen to eat of the infected grass, all the guinea-pigs were seen to eat a portion of the infected grass at each feeding. The guinea-pigs were sacrificed one at a time on the following dates.

No of guinea-pig.	Sacrificed.	Days after 1st feeding.	Days after most favorable feeding, October 31.	
1	November 29	40	28	
2	January 4	76	64	
3	January 25	97	85	
4	February 23	126	114	
5	April 2	164	152	(Became infected)
6	April 21	183	171	(Became infected)

Blocks of muscle were taken from the right and left thigh, right and left foreleg, one pectoral, abdominal muscle, diaphragm and psoas. The esophagus was also sectioned and the liver of three of the animals. The muscle was fixed in alcohol 95 per cent. and paraffine sections stained with eosin and hematoxylin were used. Two to ten sections from each block of tissue were examined carefully first with the low power and then with the high power and a mechanical stage, but nothing was found until sections of the thigh of No. 5 were examined. These sections contained one sarcosporidium resembling the one figured and described by Negri (7), and very much like the one found by the writer in man.

In No. 6, sacrificed 19 days later, another sarcosporidium exactly like the one found in No. 5 was found in sections of muscle from the thigh. When the animals were sacrificed, their muscles were carefully examined in gross for evidences of sarcosporidia resembling *Sarcocystis muris,* but nothing was ever noted at all suggestive of the latter or, indeed, of any species, although the writer was quite

familiar with the gross appearance of this parasite from his previous examinations of many infected rats. It should be remarked that it is strange that instances of rats infected with *Sarcocystis muris* have not been reported oftener when one considers the hundreds of thoussands of rats examined at plague laboratories. The explanation, no doubt, is that plague rats are examined for enlarged groin glands and for the purpose of making bacteriological examination of the spleen; in this way, the thigh muscles, which are most commonly invaded, would be overlooked.

DESCRIPTION OF SARCOSPORIDIA IN INFECTED GUINEA-PIGS.

Guinea-pig No. 5 (Fig. 4).—There is an obliquely cut parasite .111 millimeter in length, and 0.024 millimeter in width, in the interior of a muscle fiber, the diameter of which is 0.033 millimeter. The parasite is oval, and in a few sections at one end of the body of the parasite a very thin unstriated eosin staining capsule, or envelop enclosing the sporozoites can be seen. The sarcosporidium contains hundreds of minute sporozoites, which are slightly curved or banana-shaped, each one of which has a single nucleus, staining deeply with hematoxylin. The nucleus is round, oval, or oat-shaped, and occupies about one-third the space of the eosin staining sporozoite; it is placed at one end of the sporozoite. The opposite end of the sporozoite is generally acuminate. The sporozoite is 3.75 microns long and 1.0 micron wide. In most of the sections, the sporozoites are closely packed together without any arrangement, but in one section they were matted together in little groups of twenty or more (in section) suggestive of clusters in room-chambers (Kammerung), but no reticulum enclosing and separating the groups such as is seen in *Sarcocystis muris* could be detected. Another block of muscle from this guinea-pig contains many muscle fibers that have undergone a peculiar basophilic degeneration. The fibers are fibrillated and the fibrillar blocks are dissociated and separated one from another. They stain blue with hematoxylin. There is no associated leucocytic or cellular infiltration, and no evidences of myositis. No sarcosporidia could be found in sections of this block. This basophilic degeneration may have been caused by a sarcotoxin formed by the sarcosporidia such as has been studied by Laveran (9).

Guinea-pig No. 6.—A block taken from one of the thighs contains, in section, a sarcosporidium just beneath the fascia but enclosed partly within a muscle fiber. This apparently shows one of the ends of the sarcosporidium. The capsule is wrinkled, slightly shrunken, and encloses a number of sporozoites which are separated sufficiently from one another so that their morphology can be made out. No room-chambers could be detected.

Occasionally, in sections of Guinea-pigs Nos. 2, 3 and 4, a few atypical large nuclei in unusual locations were seen, which were suggestive of resting spores (sporont).

Guinea-pigs Nos. 1, 2, 3, and 4, while presenting no direct evidences of sarcosporidia, would occasionally show a few small foci of cellular infiltration within a single muscle fiber. These foci of infiltration may have replaced sarcosporidia that had died.

The results of this experiment confirm the one previously conducted by Negri (7) with guinea-pigs infected with *Sarcocystis muris*. The greater length of time required before parasites could be demonstrated in the guinea-pigs muscle may be due in some way to the fact that the guinea-pigs were more nearly in their normal environment—the tropics—and may have been slightly more resistant to infection.

It must be said that parasites in Guinea-pigs Nos. 5 and 6 were extremely sparse, and if a much greater number of sections from Guinea-pigs Nos. 2, 3, and 4 had been examined, it is possible that parasites might have been found. It was not thought necessary to do this, inasmuch as the important fact of infection had been demonstrated.

The results of the experiment then are:

The infection of guinea-pigs with a sarcosporidium derived from *Sarcocystis muris* of rats, the parasite becoming evident in the muscular tissue after a period of five months, and appearing to be undeveloped, abortive, and being microscopic in size.

A comparative examination of the sarcosporidium obtained from guinea-pigs, with the one found by the writer in man, brings out several points of similarity.

Both forms are microscopic; they apparently develop without the formation of room-chambers. Their contour, size and general ap-

pearance is about the same, and their sporozoites are practically the same size.

When the guinea-pig sarcosporidium is compared with the one from which it is derived, some remarkable alterations in morphology in the guinea-pig parasite are noted.

When tabulated, they appear as follows:

Ripe sporozoon.	From rat *Sarcocystis muris*. (mm.)	From guinea-pig (rat). Writer's experiment. (mm.)	From guinea-pig (rat). Negri's experiment. (mm.)	From man. Writer's case. (mm.)
Length	13.000[2]	0.111[3]	0.1[3]	0.084[3]
Width	0.208[2]	0.024[3]	0.03[3]	0.027[3]
Sporozoite.				
Length	0.012[4]	0.00375[3]	0.003 to 0.005[3]	0.00425[3]
Width	0.006[4]	0.001[3]	?	0.00175[3]

Beside the great reduction in size of the ripe parasite and its sporozoites in the guinea-pig sarcosporidium, there are finer changes in the sporozoite, such as staining qualities and the location of the nucleus. In the guinea-pig parasite, and in that of man, the nucleus is at one end of the sporozoite, while in *Sarcocystis muris*, studied from smears, the nucleus is nearer the middle and has a well defined nuclear membrane. However, an exact comparison cannot be made because the finer structures of the sporozoites can be seen only in stained smears and the guinea-pig parasite has been detected only in sections of tissue which are too coarse to show anything more than the size, form, and location of the nucleus.

The sarcosporidium found by the writer in man is so small, and resembles the guinea-pig parasite so closely, that one cannot escape the conviction that each is an example of an aberrant form—a sarcosporidium developing in the muscular tissues of an unusual host.

Naturally, it remains to be seen whether the guinea-pig sarcosporidia can be made to develop back into *Sarcocystis muris* and regain their normal morphology by feeding infected guinea-pigs to rats or mice. This would be an extremely difficult experiment to carry out on account of the sparseness of sarcosporidia in the guinea-pigs, and because of the many naturally infected rats.

One of the most striking things noted in this experiment was the

[2] Fresh specimen.

[3] Sections fixed in alcohol, etc.

[4] Smears stained with Hasting's stain.

extreme sparseness of the parasites in the guinea-pig, notwithstanding the enormous number of sporozoites which must have been ingested.

The mode of infection in man, in the writer's case, might be explained by his having eaten some uncooked, or insufficiently cooked, infected meat; the pig-tail, or fowl, which entered into his diet, may have been infected. So far as we know, the mode of infection is always by way of the gastro-intestinal tract, and it is not unlikely that the man's infection was by *Sarcocystis miescheriana,* or by some one of the sarcosporidia of fowl, or possibly a parasite in some lower form of life as its host.

CONCLUSIONS.

1. Guinea-pigs, naturally uninfected by sarcosporidia, were infected by feeding them with rat's muscle that was naturally infected by *Sarcocystis muris,* and by ripe mobile sporozoites from the same source. The infection was not visible grossly, but was detected upon very careful search through many sections of muscle.

2. Sarcosporidia were not found in the guinea-pigs until after an interval of 164 days from the first feeding, or 152 days after the most favorable feeding, when many teased-out mobile sporozoites were fed.

3. The prolonged period of incubation or latency, and the greater time required for infection of guinea-pigs here over that required by Negri (7) in Pavia, may be related to the fact that the experiment here was conducted in the tropics where the guinea-pig is a native.

4. Sarcosporidia of this apparently abortive type in unusual hosts cannot be specifically identified until their derivation and host relationships have been determined.

5. Morphologically, the guinea-pig sarcosporidia derived from *Sarcocystis muris* are identical with those found by the writer in the biceps of a Barbadian negro, and both probably represent abortive or aberrant forms.

BIBLIOGRAPHY.

1. Smith, Theobald, The Production of Sarcosporidiosis in the Mouse by Feeding Infected Muscular Tissue, *Jour. of Exper. Med.,* 1901, vi, 1; Further Observations on the Transmission of *Sarcocystis muris* by Feeding, *Jour. of Med. Research,* 1905, xiii, 429.

2. Darling, S. T., Sarcosporidiosis with Report of a Case in Man, *Arch. of Internal Med.*, 1909, iii, 183.
3. Kartulis, Ueber pathogenischen Protozoen bei Menschen, *Zeit. f. Hyg.*, 1893, xiii, 1.
4. Döflein, *Cent. f. Bakt.*, Abt. i, 1895, xviii, 13.
5. Braun, The Animal Parasites of Man, New York, 1906.
6. Baraban and St. Remy, *Compt. rend. Soc. de biol.*, 1894, x, 201.
7. Negri, A., Beobachtungen über Sarkosporidien, *Cent. f. Bakt.*, Orig., 1908, xlvii, 612.
8. Nègre, L., Sarcosporidiose experimentale, *Compt. rend. Soc. de biol.*, 1907, lxiii, 374.
9. Laveran, *Compt. rend. Soc. de biol.*, 1899, li, 249.

EXPLANATION OF PLATES I AND II.

FIG. 1. Rat muscle (*Mus rattus*) showing cross-section of parasite—*Sarcocystis muris.*

FIG. 2. *Sarcocystis hominis* (writer's case) section of biceps showing cross-section of parasite.

FIG. 3. *Sarcocystis hominis* (writer's case) section of biceps showing long section of parasite.

FIG. 4. Guinea-pig No. 5. Sarcosporidium derived from *Sarcocystis muris.*

FIG. 1.

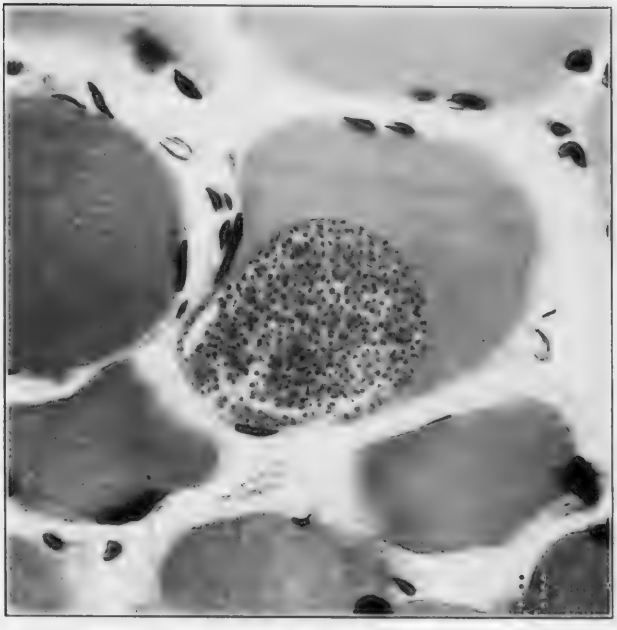


FIG. 2.

FIG. 3

FIG. 4.

Mau

RESISTANCE PRODUCED IN MICE AGAINST TRANSPLANTED CANCER BY AUTO-INOCULATION OF THE SPLEEN.[1]

By WILLIAM H. WOGLOM, M.D.

(*From the Laboratory of the Imperial Cancer Research Fund, London.*)

It has been shown by the Imperial Cancer Research Fund[2] that mouse blood injected into other mice educes within ten days a condition in these animals during which they are refractory to the inoculation and growth of some transplantable tumors. These results were confirmed by other workers, while still others were unable thus to produce resistance against the tumors with which they were working. Analyzing the results of their own experiments, the workers above referred to proved that resistance was conferred only by the cellular elements of the blood, and not at all by the serum.[3] While it had been shown in the same laboratory that young mice afford a more suitable ground for the growth of transplantable tumors than old ones, it was found that the blood corpuscles of old mice are not more active in the production of this resistant state than those of younger animals. The inoculation of corpuscles of closely allied animals, even those of the rat, will not produce an equally high refractory condition although they may appear to do so when careful attention is not given to age, weight and race of mice, to time intervals, and to the quantitative relations on which depend the reactions following the inoculation of tumors. These precautions were rigorously observed in the experiments about to be described, and these important details are given as, *e. g.*, in Fig. 1, drawn to scale from charts showing the tumors in natural size.

Schöne[4] was able to elicit resistance by the inoculation into mice

[1] Received for publication September 9, 1909.

[2] Bashford, Cramer and Murray, *Brit. Med. Jour.*, 1906, ii, 207.

[3] *Proc. of the Royal Soc.*, Series B, 1907, lxxix, 164.

[4] *Münch. Med. Woch.*, 1906, liii, 2517.

of mouse embryos, and Borrel[5] and Bridré[6] by the inoculation of liver, spleen and brain, but not of testis. Schöne's observation was confirmed and extended by the Imperial Cancer Research Fund, who, following up the analysis made in regard to the elements of the blood, showed that different parts of the embryo are variously active in producing resistance, and that the skin is the most active of them all. The refractory state which ensues upon the inoculation of normal tissues is at its height between ten and fifteen days after the inoculation.

The question naturally presents itself whether the inoculation into a mouse of its own tissue would confer the same resistance as would be followed by the introduction of the corresponding tissue of other mice. Experiment proved that it was impossible to withdraw from a mouse sufficient blood for inoculation, and it was, therefore, necessary to make use of some other tissue. The injection of spleen is known to produce a resistance as high as that which follows the inoculation of blood, and it is, in addition, an organ, the removal of which is well tolerated by the mouse.

Accordingly, a number of mice were splenectomized and the spleens emulsified by repeatedly cutting with sharp scissors—a procedure which affords a perfect emulsion without destroying the integrity of the individual cells. The spleens, thus emulsified without the addition of any extraneous fluid, were inoculated, each into its corresponding mouse. The emulsion was placed in the axilla by means of a Pasteur pipette introduced into the groin and pushed forward. Twelve days afterward the mice were inoculated in the opposite axilla, each with an accurately measured dose (0.025 cubic centimeter) of an emulsion of Tumor 63, a mammary adenocarcinoma. For controls, the same number of mice were inoculated at the same time, in the same location, and with an equal amount of the same tumor emulsion. Care was taken to have all the mice of the same weight, and, therefore, of approximately the same age.

The result of an antecedent inoculation of homologous tissue is clearly shown in Fig. 1. It is just as successful in evoking a refractory state as is previous treatment with tissue from other mice.

[5] *Bull. de l'Inst. Pasteur,* 1907, v, 497, 545, 593, 641.
[6] *Ann. de l'Inst. Pasteur,* 1907, xxi, 760.

That splenectomy, as such, has no influence on the growth of a subsequently inoculated tumor is shown by a control experiment, in which twelve days after splenectomy the mice so treated were inoculated with Tumor 63. Of these animals, 71 per cent. (5 positive

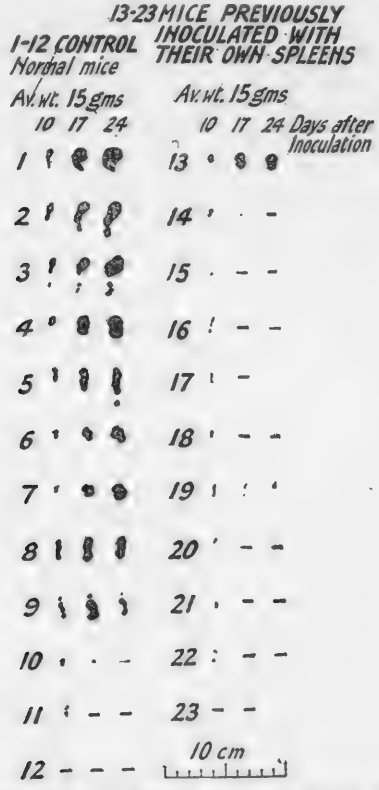


FIG. 1. Experiment 63/31D, showing that mice can be rendered resistant to inoculation of cancer by a preliminary inoculation with their own spleens. All mice were inoculated July 12, 1909, with 0.025 c.c. of Tumor 63, in the left axilla. Mice 1 to 12 are normal and untreated mice (controls). Mice 13 to 23 are splenectomized mice, inoculated with their own spleens 12 days before the inoculation of the tumor.

When I left England, a second experiment was under way, the final outcome of which Dr. Bashford has been kind enough to send me. Although the immunity gained by the mice inoculated with homologous spleen is not so striking as it was in the first experiment, it is nevertheless present.

in 7) developed tumors, and of their controls, 69 per cent. (11 positive in 16).

While it had been shown by the Imperial Cancer Research Fund that normal tissues of other mice inoculated into mice bearing transplantable tumors were powerless to effect the disappearance of these tumors, it was necessary to know what result would follow the inoculation of homologous tissue into tumor-bearing animals. Forty mice were inoculated with Tumor 63, and thirteen days later, of the thirty-six that survived, twenty were splenectomized and inoculated each with its own spleen, while the remaining sixteen were reserved as controls.

At the latest available charting, eleven days after the inoculation with the spleens, 90 per cent. of the inoculated mice had tumors, and 87 per cent. of the controls. A few of the tumors in each group were receding, but the percentage of these in the treated mice was not larger than in the controls. Thus homologous tissue inoculated after a tumor has begun to grow is without effect upon its further development. The details of this experiment are given in Table I.

TABLE I.

	Number inoculated with tumor.	Average weight.	Survived.	Positive.	Per cent.
Splenectomized and inoculated with their own spleens after tumors had developed.	20	10.3 gm.	11	10	90
Controls.	16	10.5 gm.	16	14	87

EXPERIMENT 63/28B. All mice inoculated July 30, 1909, in right axilla with 0.025 c.c. of an emulsion of Tumor 63. Twenty of them have been splenectomized August 8, 1909, and inoculated with their own spleens.

Michaelis[7] found that cancer cells killed by chloroform or heat lost their power of inducing resistance. Inoculation of material heated to 65° C. in some cases seemed to produce hypersensibility. Flexner and Jobling[8] record a similar result for their rat tumor. Unpublished experiments by Haaland, referred to by Bashford[9] in his address to the International Congress at Buda-Pest, have shown that complete disintegration of tumor or normal cells, by any one

[7] *Zeit. f. Krebsforsch.*, 1907, v, 193.

[8] *Proc. of the Soc. for Exper. Biol. and Med.*, 1907, v, 16.

[9] *Med. Record*, 1909, lxxvi, 381.

of a variety of physical agents, robs them entirely of the power to induce resistance. In some cases, animals vaccinated with killed tissues are found to be distinctly more suitable for inoculation with tumors than normal animals. It was of interest to see whether homologous splenic tissue would behave in a parallel manner under these conditions also, and an experiment was performed for this purpose. The spleens of seventeen splenectomized mice were inoculated into the same animals after having been crushed and frozen and thawed three times. Fourteen days later the mice were inoculated in the opposite axilla with 0.025 cubic centimeter of an emulsion of Tumor 63. Sixteen normal mice of the same weight were inoculated in the same way as controls.

TABLE II.

	Number Inoculated.	Survived.	Average Weight.	Positive.	Negative.	Per cent.
Control.	16	14	12.8 gm.	13	1	93
Splenectomized.	17	14	12.2 gm.	12	2	86

EXPERIMENT 63/33B. All mice inoculated in left axilla August 13, 1909, with 0.025 c.c. of tumor emulsion (63).

The protective action produced by homologous undamaged spleen was completely abolished. Conclusive evidence of distinct hypersensibility could not be obtained, but the experiments are being continued.

The homologous tissues of the mouse, therefore, behave in the same way as heterologous mouse tissues with respect to the production of resistance to cancer. This result, in itself sufficiently striking, illustrates clearly the extreme delicacy of the reactions in play.

In conclusion, I wish to express my thanks to the Executive Committee of the Imperial Cancer Research Fund for their courtesy in extending to me the privileges of their laboratory, and to record my indebtedness to Dr. E. F. Bashford, the Director of the laboratory, and to his colleagues Dr. Murray, Dr. Haaland, Mr. Bowen and Dr. Russell.

A portion of the expenses of this work was borne by the Cancer Research Fund of the College of Physicians and Surgeons, New York.

THE RESPIRATORY CHANGES OF PRESSURE AT THE VARIOUS LEVELS OF THE POSTERIOR MEDIASTINUM.[1]

By S. J. MELTZER and JOHN AUER.

(From the Department of Physiology and Pharmacology of the Laboratories of the Rockefeller Institute for Medical Research, New York.)

PLATE III.

INTRODUCTION.

Some pathological phenomena manifest a predilection for certain parts of the lungs. Emphysema, for instance, occurs preferably in the peripheral parts of that organ. Tuberculosis, on the other hand, shows a predilection for the apices. Furthermore, in experimental pneumonoconiosis, India ink or ultramarin seem to lodge first in the cranial part of the upper lobes. Are these preferences founded on, or at least favored by, some differences in the respiratory capacity of the various parts of the lungs? For the preference of tuberculosis, such an assumption was made indeed by various clinical writers. In the prebacterial period, it was simply assumed that the apices of the lungs participate in a lesser degree in the respiratory movements and that their ventilation is, therefore, not as satisfactory and the resistance to disease not as good as that of the rest of the lungs. However, with the advent of the tubercle bacillus, especially from the point of view of the theory of the aërogenous origin of tuberculosis, such an assumption manifestly made matters even more difficult; the diminished inspiration in the apex would be rather a hindering than a favoring factor in the invasion of the bacilli. To meet this difficulty Hanau[2] tried to adapt the hypothesis to the new requirement by assuming that the inspiration is better in the apex than in the rest of the lung, but that the expiration is not as good. Such an arrangement would indeed have the tendency to retain easily some of the invading bacilli. But Meltzer[3] called

[1] Received for publication September 17, 1909.

[2] Hanau, *Zeit. f. klin. Med.*, 1887, xii, 1.

[3] Meltzer, *Jour. of Physiol.*, 1892, xiii, 218.

attention to the intrinsic impossibility of such a hypothesis. No part of the lung can persist when air enters into its vesicles with better facilities than it can leave them; the vesicles would break in no time. Furthermore, the question arose as to the correctness of the assumption that the apices participate in the act of respiration in a manner different from the rest of the lungs. What basis was there for such an assumption? The question whether all parts of the lungs take an equal share in the act of respiration is a physiological problem. The discussion of this problem in the physiological literature was only very scanty, but the opinion of the few writers who have dealt with that subject was rather the reverse of that of the clinicians and the pathologists. Rosenthal[4] states that despite the fact that the greatest motion of the lungs occurs at their lower (caudal) part, each vesicle of the entire lung increases (or collapses) in the same degree. He compares the lung to a strip of elastic rubber, the one end of which is fixed, while the other end is stretched. The degree of stretching is the same at all parts of the band, although the visible motion seems to take place only at the stretched end. These, however, were only *a priori* opinions which were not supported by any experimental tests. Meltzer tried to approach this subject by the experimental method. The result of this investigation was published seventeen years ago.[5] The most satisfactory answer to the question whether all parts of the lungs respire in the same manner would be obtained if the intra-pulmonary pressure prevailing in all parts of the lungs could be studied and compared. This answer is impossible, at least for the present. The next best method would be a comparison of the intrathoracic pressure prevailing in all parts of the thoracic cavity during the respiratory changes. An attempt to study it in the pleural cavity by the methods then at hand failed. The posterior mediastinum was then attacked. A comparison of the pressures prevailing at the various heights of the esophagus gave unsatisfactory results. Meltzer finally settled upon a new path for exploration of the respiratory pressure in the posterior mediastinum; it is the path which is sometimes unfortunately selected by the retropharyngeal abscess.

[4] Hermann's Handbuch der Physiol., 1882, iv, pt. 2, 180.

[5] Meltzer, *loc. cit.*

A calibrated narrow tube, one end of which was connected with a manometer or a kymograph, was carefully and slowly pushed behind the esophagus down the entire depth of the posterior mediastinum. This was done in rabbits. These experiments gave definite and even surprising results. In the upper part of the thorax the inspiratory decrease of pressure was very little marked. Testing if further down, the decrease of pressure during inspiration increased either gradually or abruptly. In some thirty per cent. of the experiments, in pushing the tube down a short section was encountered in which the inspiratory changes became insignificant again. In the last (caudal) third of the mediastinum, the inspiratory decrease of pressure was in all experiments well marked and of uniform extent throughout the entire section. In many of the experiments the lungs were watched through a window made in the intercostal muscles. It was thus established that the sudden rise of the negative pressure in the mediastinum was not coincident with any change in the position of the lung in the pleural cavity.

It was thus demonstrated that the upper (cranial) part of the mediastinum reacts differently to the effect of an inspiration than the lower (caudal) part. While this seemed to be a well secured fact, Meltzer nevertheless hesitated to commit himself to the interpretation that the diminished inspiratory negative pressure in the upper part of the mediastinum means positively a diminished intrapulmonary pressure in the upper part of the lungs during inspiration. He calls attention to the presence in the upper third of the mediastinum of such bulky organs as the venæ cavæ, the aortic arch, the tracheal bifurcation, etc., which may account for the lagging behind of this part of the mediastinum in the adequate response to the inspiration. Meltzer concludes by saying: "Although it seems to me that my experiments on the mediastinum make it indeed probable that the apices and the back part of the upper third of the lungs do not participate in the breathing so largely as the other parts of the lungs, in consideration of the importance of this conclusion, we should not consider it as proven until it has been tested by experiments on the pleural cavity directly, or within the lungs themselves."

Recently the subject of the respiratory changes of pressure in the

various parts of the lungs has been dealt with extensively by N. Ph. Tendeloo in journal articles, addresses and in a book especially devoted to that question.[6] Tendeloo is of the opinion that any change in the intrapulmonary pressure of one part of the lung does not necessarily cause an intrapulmonary pressure in any other part of it, and he is emphatic in the assertion that the cranial and paravertebral parts of the lungs take a lesser share in the respiration than the other sections of the lungs. He supports his views by extensive arguments based on a fine analysis of known physical, anatomical and pathological facts. As to the experimental evidence of a physiological nature, Tendeloo has at his disposal only the above mentioned results of Meltzer, which, in disagreement with this author, Tendeloo thinks are absolutely convincing that the respiratory changes in the intrathoracic pressure are not the same in the entire thoracic cavity, but are diminished in the superior-posterior direction.

While the present writers were also inclined to believe in the correctness of the hypothesis that the normal respiratory changes of pressure are not the same in all parts of the cavity, they were of the opinion that, despite the experiments of Meltzer and the interesting arguments of Tendeloo, this hypothesis can not be considered as proven and that further investigations contributing to the elucidation of that problem is still very desirable. By bringing forward step by step some well established new facts, be they ever so small, it may so happen that the problem will be solved some day on a safe basis. In the following we intend to offer such an experimental contribution of modest dimensions.

THE EXPERIMENTAL CONTRIBUTION.

Method.—It has already been mentioned that the esophagus offers a natural path for the study of the intrathoracic pressure. This method, however, was discarded by different investigators for various reasons. In the first place, the thickness of the wall of the

[6] Tendeloo, Studien über die Ursachen der Lungenkrankheiten, Wiesbaden, 1902. Address at the meeting of the Gesellschaft Deutscher Naturforscher und Aerzte, Dresden, 1907. International Congress of Tuberculosis, Washington, 1908. *Med. Klin.*, 1909, v, 1300.

esophagus does not permit one to draw a conclusion regarding the actual respiratory pressure in the thoracic cavity. Secondly, the presence of the tube within the esophagus acts as a foreign body and causes irregular local contractions of that tube, thus obscuring the respiratory changes. Occasional peristaltic contractions due to deglutition add to the confusion. Furthermore, the occasional opening of the cardia and regurgitation of air or liquid into the esophagus is another disturbing factor. Finally, the presence of mucus in the esophagus coming from above might cause an obliteration of the "windows" in the tube within the esophagus, or might cause a stickiness of the rubber capsule, if such is used, and hamper its responsiveness to the changes of the respiratory pressure.

Notwithstanding these objections, we decided to try the esophageal method again. The first objection does not hold good for the present experiments, since we did not study the actual pressures obtaining within the thoracic cavity; we intended only to compare the pressures prevailing in the upper and the lower parts of the posterior mediastinum. For this purpose the thickness of the wall of the esophagus can be no obstacle as long as the thickness is practically the same throughout the thoracic part. As to the other objections, we met them by the following arrangements: In the first place both vagi were cut in the neck which eliminated the tonicity and possible contractions of the esophagus. Then the esophagus was tied in the upper part in the neck and beneath the diaphragm, thus preventing the entrance of mucus from above and of gas from below. The esophagus presented then a closed sausage-shaped soft bag fitted into the posterior mediastinum and readily responding to the changes of pressure within it. Through a small opening made beneath the cervical ligature a catheter was introduced into the esophagus and again a ligature was tied around the esophagus beneath the opening in order to prevent the entrance of air. This ligature, however, was not made too tight to prevent the catheter from moving up and down. The side openings of the inner end of the catheter were either free or had a small bag of thin rubber, a thin finger cot, tied around them. The outer end of the catheter was connected by means of rubber tubing with a Marey's tambour which transmitted the respiratory changes of pressure within the esoph-

agus to a kymograph in the usual manner. A T-tube connected with a stopcock was inserted in the tubing for the regulation of the pressure within the system, when necessary.

Dogs were used in this series of experiments. On account of the section of the vagi the animals were tracheotomized. They were narcotized with morphine and ether; in some cases magnesium sulphate, by intramuscular injection, was given instead of ether. It is important for the success of the experiment that the respirations should be neither too shallow nor too deep (dyspneic). On account of the section of the vagi, respiration is apt to be too deep. A carefully conducted anesthesia helps greatly in regulating the respiration.

Results.—The results which were obtained were strikingly uniform. The increase of the negative pressure during inspiration was studied extensively. At the beginning of the study of a new level of the esophagus the inspiration started from the atmospheric pressure. This was accomplished by opening the stopcock connected with the tubing and closing it during the expiratory pause. In this way we obtained a series of tracings presenting normal inspirations for each level of the esophagus. At the autopsy, the location of these levels and their relations to the neighboring organs were established.

In general, the esophagus may be divided into three sections. In the uppermost part of the first section the inspiratory undulations are quite small, but they increase, more or less gradually, with the depth of that section. In the next section the undulations become again very small, but increase more or less gradually when the end of that section is approached. Then follows the third section in which the undulations abruptly become large and practically remain so to the end of the section. Any inspiratory undulation of this section is distinctly larger than any undulation in the first section.

The first section extends from the thoracic aperture to the level corresponding to the bifurcation of the trachea. The second section extends from the bifurcation to a level corresponding to about the apex of the heart. The third section extends to the diaphragm. When the small balloon approaches the passage of the esophagus through the hiatus of the diaphragm, the undulations become very

small, even if the cardia is not tied. In other words, the reduction in pressure within the thoracic esophagus due to normal inspiration is small at the level of the apex, but increases gradually until it reaches the level of the bifurcation, where it becomes considerably reduced again or may even disappear completely. While the balloon passes the level of the heart, the inspiratory reduction of pressure may more or less gradually increase again. However, as soon as the balloon emerges from opposite the apex of the heart the inspiratory negative pressure reaches its maximum abruptly and remains so until the diaphragm is encountered. In brief, the inspiratory changes within the thoracic esophagus differ greatly with the different levels; the changes are most pronounced at the caudal part and are only moderate opposite the apex of the lungs, while opposite the tracheal bifurcation and the heart the changes are very small.

These differences in the inspiratory changes remain practically the same even with a one-sided pneumothorax; with a double open pneumothorax there are practically no respiratory changes within any part of the esophagus.

With open double pneumothorax and artificial respiration the inspiratory changes are the same in the upper and the lower part of the esophagus, but are considerably lessened in the middle part. The increase of pressure is, of course, positive with each inspiration, when the artificial respiration is carried out by blowing air into the trachea. In one case, artificial respiration was carried out immediately after death by rhythmically pulling the diaphragm (liver) downward. In this case the inspiratory changes of pressure within the esophagus were the same as in normal respiration.

In dyspnea, when the inspirations are very deep, there is practically no difference in the respiratory changes at the various levels of the esophagus.

The expiratory changes within the esophagus were not studied with the same attention as the inspiratory changes. From what we have observed we may conclude that all that was said for the inspiratory pressure holds good also for the expiratory changes in pressure at the various levels of the esophagus.

DISCUSSION.

The esophagus is a natural path. In the way it was prepared by us it presents a closed canal with passive responsive membranes fitted lengthwise into the posterior mediastinum. There is no room for doubt that the changes of pressure which were found to occur at the various levels represent actual conditions within the esophagus and are not accidental artefacts. It was found that the changes of pressure accompanying the normal respiratory phases were invariably smaller in the upper than in the lower part of the thoracic esophagus and that at a certain middle part the changes were smaller than at either end. That demonstrates, in the first place, that in normal respiration each section of the esophagus presents a separate compartment, and respiratory changes in pressure in this compartment are not transmitted, at least not in full force, to any distant part of the cavity of the esophagus. We may, therefore, take the changes of the respiratory pressure which take place in each segment of the esophagus as an expression of the respiratory changes which take place in the corresponding segment of the adjacent cavity outside of it, that is in the first place in the various segments of the posterior mediastinum. Our experiments, therefore, prove first, that the respiratory changes in the posterior mediastinum are much larger in the lower than in the upper part. This corresponds exactly with the findings of Meltzer, who studied the mediastinum by an artificial path which invited the objection that the finding might have been an artefact. The full agreement between the two findings gives the result the assurance of a well-established fact.

It was found further that in all cases of normal respiration at the beginning of the middle third of the esophagus the respiratory changes were the lowest. In Meltzer's investigation this occurred in only about thirty per cent. of the experiments. The discrepancy may be due to the fact that the artificial path made in the last mentioned experiment varied perhaps in its course; or it may be because in rabbits, upon which these experiments were made, the conditions differ from those obtained in dogs. At any rate, it is a well-established fact that in dogs, at the level of the bifurcation and a little further down, the respiratory changes are less manifest than at any other place within the thoracic esophagus.

We are then sure that within the posterior mediastinum the respiratory pressure varies in various sections of the mediastinum. What is the cause of these variations? Can this mean that there is a corresponding variation in the degree of respiration in the parts of the lung adjoining these sections? With reference to the reduced changes in the respiratory pressures in the section of the mediastinum occupied by the tracheal bifurcation and the heart, we may readily answer that it is not due to a reduced respiration in the corresponding parts of the lungs. It is rather probable that the inelastic bulky tissues of the bifurcation and the heart which separate the esophagus (and posterior mediastinum) from the corresponding part of the lungs prevent the full transmission of the respiratory changes. This plausible interpretation is borne out by the fact that even the inspiratory positive pressure produced by the intratracheal artificial respiration is not well transmitted at the level of the esophagus adjacent to the bifurcation and the heart; it can not be assumed that these parts of the lungs can not be reached by the artificial respiration as well as any other part.

It is different, however, with regard to the diminished respiratory changes in the esophagus and mediastinum in the proximity of the upper part of the lung. Here the lung is not separated from the mediastinum by any specially resistant tissues and here we find that in artificial respiration the respiratory changes are in the upper part, indeed, of the same magnitude as in the lower part. We, therefore, assume that the difference in the respiratory changes between the upper and the lower part in the mediastinum has its origin in the fact that the respiratory changes within the lungs themselves are in the upper part not as extensive as in the lower part. This difference cannot be due to any difference in the elasticity in the different parts of the lungs, since artificial respiration distends all parts of the lungs apparently in the same degree. It is rather probable that the change of the respiratory pressure which is brought about by the change of position of the diaphragm and the ribs, and which begins at the caudal part of the lungs, loses a part of its force on its way to the upper part.

That in very deep, dyspneic respirations the changes in pressure become equal at all levels of the esophagus finds its explanation in

the plausible assumption that by very deep inspirations the lumen of the esophagus becomes sufficiently opened up so as to convert the entire thoracic esophagus into a well connected canal.

SUMMARY.

The changes of the respiratory pressure within the esophagus, as well as within the posterior mediastinum, differ greatly in their various levels. They are best in the section between the heart and the diaphragm; they are moderate from the upper aperture of the thorax to above the tracheal bifurcation, and they are very much reduced in the region of the bifurcation and the heart. In the latter case the normal respiratory changes are reduced in their transmission to the mediastinum by the intervention of the inelastic tissues of the bifurcation and the heart. The difference in the changes of the respiratory pressure between the lower and the upper part of the mediastinum is due to a difference in the respiratory changes of pressure between the lower and upper parts of the lungs. The changes in the respiratory pressure which begin in the lower part of the lungs lose some of their force on their way to the apices of the lungs. In artificial respiration by intratracheal insufflation there is no difference in the respiratory pressures between the upper and the lower parts of the lungs.

This investigation supports the view, frequently entertained by clinicians, that the respiratory changes in the apices of the lungs are not as good as those of the rest of the lungs, and it disproves the claim of some physiologists that a decrease or increase of pressure at any part of the lungs must be equally distributed through all parts of the lungs, a claim based upon merely *a priori* physical considerations.

EXPLANATION OF PLATE III.

This tracing shows the respiratory changes of pressure obtained by means of a balloon-tipped sound from different levels of a dog's esophagus. With each change of position of the sound, atmospheric pressure, shown by the straight horizontal line at the top of the curve, was reëstablished during expiration; above this line is positive pressure, below is negative. Downstroke represents inspiration. Time is marked in seconds.

March 26, 09 - JK Young.
Auricle.
8 cm = Right heart loop
10 cm = mean Mid Limb (short class)
12 cm = 4 cm above Auriculo-V
14 cm = 2 cm "
16 cm = at Auriculo-ventricular
Mid
8cm
10 cm
12cm
14cm
16cm
2cm
4cm
6cm

THE EXCRETION OF CALCIUM AND MAGNESIUM AFTER PARATHYROIDECTOMY.[1]

By JEAN V. COOKE, M.D.

Instructor in Pathology, University and Bellevue Hospital Medical College, New York.

(*From the Carnegie Laboratory, University and Bellevue Hospital Medical College, New York.*)

Within the past few years evidence has gradually accumulated, both direct and indirect, indicating some definite relation, the nature of which is as yet quite obscure, between the kations of calcium salts and certain forms of muscle irritability. The observations recorded may be summarized as follows: (1) Abnormal muscular twitchings and contractions may be brought about in an organism by a reduction in the proportion of calcium (or magnesium) in the muscles or blood (J. Loeb); (2) the injection into the animal body of any salt liable to precipitate calcium causes twitchings of the muscles (J. Loeb); (3) in tetany of children, the calcium content of the brain has been found lowered (Quest); (4) infantile tetany has shown marked improvement following the administration of calcium salts (Netter); (5) soluble calcium salts exert a wonderful curative effect on the tetany in dogs following parathyroidectomy (MacCallum and Voegtlin).

In a recent communication MacCallum and Voegtlin[2] describe a series of experiments in which they found an increased excretion of calcium in the urine and feces of parathyroidectomized dogs during tetany. They also found a decrease in the calcium content of the brain and blood of dogs in tetany as compared with normal

[1] Aided by a grant from the Rockefeller Institute for Medical Research. Received for publication October 6, 1909.

[2] MacCallum, W. G., and Voegtlin, C., On the Relation of Tetany to the Parathyroid Glands and to Calcium Metabolism, *Jour. of Exper. Med.*, 1909, xi, 118. (This article contains a review of the literature.)

dogs; an increased output of nitrogen and ammonia in the urine, and an increased content of ammonia in the blood. The conclusion is drawn that the parathyroid secretion in some way controls the calcium metabolism in the body, that in the absence of this secretion the calcium for some reason is excreted in excess, and that the tetany, which has been proved to be of nervous origin, is due to a hyperexcitability of the nerve cells, the result of calcium starvation.

The investigation which I here present was undertaken for the purpose of determining whether an increased excretion of calcium during tetany would be more apparent if the observations were extended over a longer period in animals receiving a calcium-poor diet, and also, to determine whether the increased salt elimination described was confined to calcium or included also magnesium. The quantitative estimation of the fixed alkalis would also have been important, but the time at my disposal did not permit it.

The observations fall into two groups, first, the excretion of calcium and magnesium in animals on a constant diet and in those fasting; second, the excretion of calcium and magnesium in animals on a constant diet followed by a fasting period during which the parathyroids were removed. The first group serves as a control for the second, and in two instances both these procedures were carried out on the same animal so that there could be no possibility of an individual variation. The necessity for the fasting was two-fold: (1) During the tentany, animals frequently refuse or are unable to eat, and even when the difficulties of administering solid food artificially are overcome, the animals will not retain it, so that the conditions do not remain absolutely constant; (2) during the fasting the salt excretion is considerably reduced and it was thought that at the constant low level of salt elimination reached, variations would be more apparent.

In order to be able to procure feces for analysis at sufficient intervals during the course of the experiments, it was necessary to prolong the observations over considerable periods of time. Several determinations of the calcium and magnesium content of the brain were made in normal dogs and those dying in tetany.

The animals used were female dogs weighing from 6 to 9 kilo-

grams. They were kept in metabolism cages under as constant and comparable conditions as possible. For several weeks before the beginning of the observations they were fed a constant weighed amount of beef heart, cracker meal and lard and were practically in nitrogenous equilibrium. When fed during the experiments they were given this same diet, which on analysis contained only a small quantity of calcium and magnesium. During all the experiments the animals were catheterized every morning at the same hour. To obtain constant amounts of urine 350 cubic centimeters of distilled water was given daily by the stomach tube. All operative procedures were carried out under complete ether anæsthesia.

Chemical Methods.[3]—The total daily urine was evaporated to dryness and ashed in a quartz dish at low temperature with addition at the end of some nitric acid and occasionally a small amount of potassium nitrate. The ash was dissolved in hydrochloric acid, made alkaline with ammonium hydrate, faintly acidulated with acetic acid and the calcium precipitated from the solution while boiling by the addition of ammonium oxalate. The oxalic acid in the collected calcium oxalate was liberated by the addition of sulphuric acid and determined by titration against N/20 potassium permanganate solution.

The filtrate from the calcium oxalate was made alkaline with ammonium hydrate and the magnesium precipitated by the addition of a solution of ammonium acetate and sodium phosphate. The ammonium magnesium phosphate was then filtered off, ignited and weighed as the pyrophosphate. In a certain number of instances the urine was divided into two portions, each of which was carried through separately in order to control the technique. The variations were quite minute.

The feces were incinerated in the same manner as the urine, iron being removed as the phosphate from the acetic acid solution. Fractions of the dissolved ash of the feces were analyzed in the same manner as the urine.

[3] I am greatly indebted to Professor John A. Mandel for many valuable suggestions and general direction of the chemical work here reported. The methods used in the determination of calcium and magnesium are well known procedures and are given merely for the sake of completeness.

TABLE I.

Calcium and Magnesium Content of 100 Grams of Dried Brain.

Normal Dogs.

	Calcium.	Magnesium.
No. 4	0.0202 gr.	0.0606 gr.
No. 5	0.0193 gr.	0.0597 gr.
No. 16[4]	0.0201 gr.	0.0600 gr.

Tetany Dogs.

	Calcium.	Magnes um.
No. 3	0.0281 gr.	0.0609 gr.
No. 15	0.0297 gr.	0.0575 gr.

TABLE II.

Showing Effect of Fasting on Excretion of Calcium and Magnesium. Dog No. 6. Standard diet January 1 to 31; fasted February 1 to 14; 350 c.c. distilled water daily; weight at beginning of experiment 6.6 kg.

Date.	Urine.		Feces.	
	Calcium.	Magnesium.	Calcium.	Magnesium.
January 19	0.0112			
" 20	0.0113			
" 21	0.0090		0.1440	0.0388
" 22	0.0116			
" 23	0.0169	0.0318	0.2041	0.0623
" 24	0.0101	0.0264	0.1252	0.0437
" 25	0.0124	0.0364	0.0721	0.0371
" 26	0.0116	0.0421		
" 27	0.0115	0.0419		
" 28	0.0116	0.0420	0.2834	0.0797
" 29	0.0095	0.0335		
" 30	0.0101	0.0378		
" 31	0.0095	0.0401	0.1368	0.0757
February 1	0.0057			
" 2	0.0031		0.0436	0.0060
" 3	0.0035			
" 4	0.0039			
" 5	0.0031	0.0070		
" 6	0.0030	0.0071		
" 7	0.0032	0.0079		
" 8	0.0024	0.0062		
" 9	0.0032	0.0095		
" 10	0.0027	0.0068		
" 11	0.0033	0.0067	0.2302	0.0147
" 12	0.0024	0.0074		
" 13	0.0035	0.0084		
" 14	0.0028	0.0060		

Explanation of Table II.—The calcium and magnesium excreted in the urine is fairly constant on the standard diet, and after decreasing for the first two days of fasting, reaches a low level that remains constant during the rest of the experiment. The feces show a marked decrease also in the calcium and magnesium.

[4] Dog 16 had been starved for two weeks to see if fasting had any influence on the salt content.

For the estimations of calcium and magnesium in the brain, the entire brain was dehydrated with alcohol on a water-bath, and dried at 100° C. to approximately constant weight, ashed, and the salts estimated as detailed above. The tabulated results are calculated for 100 grams of dried brain. In the tables, the results are all expressed as calcium and magnesium in grams.

TABLE III.

Showing Effect of Fasting on Excretion of Calcium and Magnesium. Dog No. 6 (continued). Standard diet February 15 to 25; fasted February 26 to March 3; 350 c.c. distilled water daily.

Date.	Urine.		Feces.	
	Calcium.	Magnesium.	Calcium.	Magnesium.
February 15	0.0065	0.0202	0.1563	0.0210
" 16	0.0091	0.0277	0.0985	0.0260
" 17	0.0090	0.0193		
" 18	0.0073	0.0153	0.0695	0.0421
" 19	0.0052			
" 20	0.0053		0.1166	0.0349
" 21	0.0058	0.0167	0.1357	0.0219
" 22	0.0055	0.0175	0.2072	0.0388
" 23	0.0057	0.0137		
" 24	0.0053	0.0155		
" 25	0.0055	0.0164	0.2313	0.0442
" 26	0.0042	0.0163		
" 27	0.0037	0.0069		
" 28	0.0029	0.0044		
March 1	0.0032	0.0049		
" 2				
" 3	0.0033	0.0051	0.3892	0.0475

Explanation to Table III.—This is a second fasting period on the same animal (Dog 6). On the resumption of the diet after the first period of fasting, the daily amount of calcium and magnesium in the urine immediately rose but the level reached was much lower than that found before the animal had been starved. This is probably due to the fact that the diet contains very little calcium and magnesium. The decrease in these metals in the urine and feces during this second starvation period is quite similar to the first (Table II).

TABLE IV.

Showing Effect of Parathyroidectomy on Excretion of Calcium and Magnesium. Dog No. 6 (continued). Standard diet March 4 and 5; fasting began March 6; 350 c.c. distilled water daily.

Date	Urine.		Feces.		
	Calcium.	Magnesium.	Calcium.	Magnesium.	
March 4	0.0039	0.0099			
" 5	0.0064	0.0172			
" 6	0.0040	0.0078			
" 7	0.0045	0.0079	0.1857	0.0514	
" 8	0.0060	0.0075			
" 9	0.0044	0.0074	0.0500	0.0204	Operation.
" 10	0.0043	0.0076	0.0102	0.0005	
" 11	0.0031	0.0162			
" 12	0.0038	0.0240			Tetany slight.
" 13	0.0044	0.0341	0.1197	0.0110	" "
" 14	0.0049	0.0372			" "
" 15[5]	0.0039		0.1706	0.0434	No tetany.
" 16[5]	0.0040		0.2061	0.0671	No tetany.
" 17	0.0043	0.0053	0.0409	0.0051	Tetany slight.
" 18	0.0058	0.0070	0.0175	0.0075	Tetany severe.
" 19	0.0043	0.0140	0.1535	0.0352	
" 20[6]	0.0075	0.0156	0.1828	0.0273	
" 21	Died 3 P. M.				Tetany severe.

Explanation of Table IV.—On March 9, four parathyroids were removed. Slight fibrillary twitchings of the tongue were observed on March 12, 13 and 14, but no other signs of tetany. The dog was fed the standard diet on March 15 and 16, no tetany being noted on those days. On March 17, however, were seen very distinct twitchings of the tongue and shoulder muscles and the next day (March 18) the animal developed a very severe tetany with tachypnea, tachycardia and convulsive twitchings of the muscles. Calcium lactate equivalent to 0.252 gm. of calcium was given intramuscularly on this date, and the tetany disappeared, but on March 21 another severe attack was fatal. It is interesting to note the progressive increase of the magnesium of the urine beginning on March 11 *before* any tetany could be detected. There is no increase in the calcium of the urine. The calcium and magnesium of the feces are not noticeably increased except on March 15 and 16, which increase is probably due to the fact that the animal was fed on these days. Note that the calcium injected on March 18 appeared, or can be accounted for almost entirely, in the feces and not in the urine.

[5] Fed standard diet.

[6] Urine contaminated with feces.

TABLE V.

Showing Effect of Fasting on Excretion of Calcium and Magnesium. Dog No. 9. Standard diet March 20 to April 19; fasted April 20 to 26; 350 c.c. distilled water daily; weight at beginning of experiment 9.1 kg.

Date.	Urine.				Feces.	
	Amount in c.c.	Specific gravity.	Calcium.	Magnesium.	Calcium.	Magnesium.
April 13	560	1014	0.0144	0.0231		
" 14	600	1014	0.0212	0.0285		
" 15	550	1015	0.0136	0.0210	3.4537	0.2001
" 16	540	1014	0.0151	0.0232		
" 17	525	1010	0.0066	0.0091		
" 18	600	1017	0.0255	0.0332	1.7575	0.1389
" 19	510	1013	0.0141	0.0188		
" 20	500	1008	0.0209	0.0242	1.6264	0.1006
" 21	375	1007	0.0092	0.0116		
" 22	400	1006	0.0085	0.0094		
" 23	450	1009	0.0105	0.0193		
" 24	350	1009	0.0063	0.0120		
" 25	350	1007	0.0071	0.0105		
" 26	340	1010	0.0042	0.0059	0.4046	0.0169

Explanation of Table V.—This table is similar to Tables II and III and shows the decreased excretion of calcium and magnesium in the urine and feces during starvation. The amount and specific gravity of the urine are decreased also during the fasting period.

TABLE VI.

Showing Effect of Parathyroidectomy on Excretion of Calcium and Magnesium. Dog No. 9 (continued). Standard diet April 27 to May 3; fasting began May 4; 350 c.c. distilled water daily.

Date.	Urine.				Feces.		
	Amount in c.c.	Specific gravity.	Calcium.	Magnesium.	Calcium.	Magnesium.	
April 27	420	1005	0.0071	0.0121			
" 28	475	1016	0.0105	0.0149			
" 29	625	1012	0.0124	0.0189	0.6118	0.0427	
" 30	610	1013	0.0174	0.0240	0.3684	0.0380	
May 1	570	1013	0.0182	0.0203			
" 2	535	1012	0.0123	0.0175	0.8458	0.0862	
" 3	540	1015	0.0155	0.0197	0.3252	0.0421	
" 4							
" 5	420	1008	0.0076	0.0120	0.8094	0.0483	
" 6	355	1008	0.0038	0.0091	0.1324	0.0086	
" 7	345	1007	0.0031	0.0074			Operation.
" 8	340	1012	0.0069	0.0270			
" 9	330	1018	0.0057	0.0080			Tetany severe.
" 10	320	1015	0.0041	0.0187			Tetany slight.
" 11	330	1024	0.0059	0.0346			Tetany severe.
" 12	210	1002	0.0020	0.0087			Tetany slight.
" 13	215	1034	0.0063	0.0392	0.2964	0.0153	Tetany moderate.
" 14[7]	50	1043	0.0038	0.0078			Tetany severe.

[7] Dog died 3:30 p. m. This is a six-hour specimen.

Explanation of Table VI.—Parathyroidectomy May 7, four parathyroids being removed. Tetany developed on the second day. On the intervening day, the magnesium of the urine was markedly increased and the calcium showed a slight increase as compared with the preceding period of fasting. After the development of tetany the magnesium increase was maintained, if averages for the entire period are taken. The calcium elimination for the same period shows no increase. The specific gravity of the urine after the development of tetany was much higher except on one day (May 12). A decrease in the volume of the urine occurred during the last few days of the experiment. In the feces, the calcium is slightly less than during the control period (Table V), and the magnesium about the same.

TABLE VII.

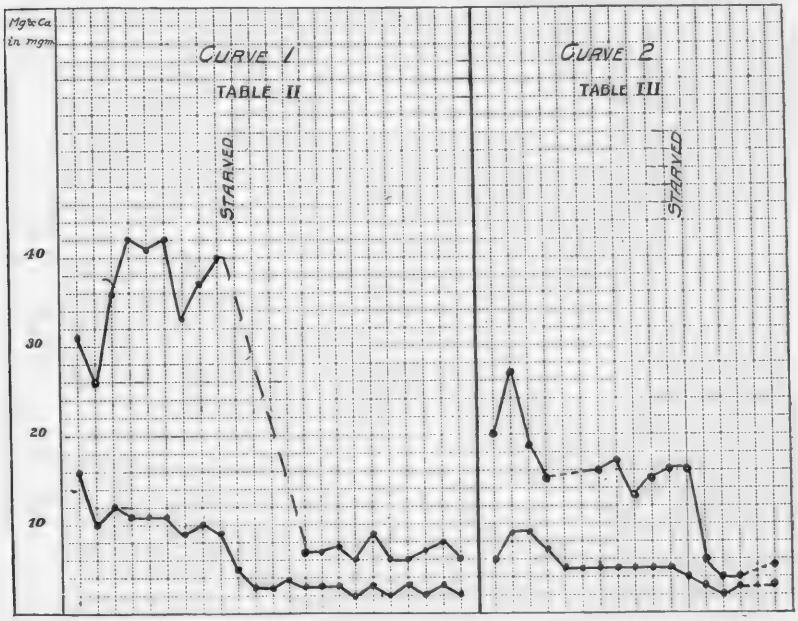
Showing Effect of Parathyroidectomy on Excretion of Calcium and Magnesium. Dog No. 10. Standard diet May 1 to 20; fasting began May 21; 350 c.c. distilled water daily; weight at beginning of experiment 6.7 kg.

Date.	Urine.				Feces.		
	Amount in c.c.	Specific gravity.	Calcium.	Magnesium.	Calcium.	Magnesium.	
May 15	355	1016	0.0096	0.0140	0.2272	0.0906	
" 16	480	1015	0.0099	0.0153			
" 17	515	1013	0.0096	0.0146			
" 18	445	1014	0.0103	0.0188			
" 19	550	1016	0.0095	0.0179	0.1333	0.0675	
" 20	430	1015	0.0116	0.0190	0.1090	0.0301	
" 21	430	1009	0.0052	0.0099			
" 22	390	1008	0.0047	0.0078			
" 23	375	1007	0.0050	0.0078			
" 24	395	1007	0.0044	0.0087			
" 25	260	1013	0.0053	0.0161	0.1919	0.0348	Operation.
" 26	270	1016	0.0061	0.0190			Tetany severe.
" 27	Died 1 P. M.						

Explanation of Table VII.—Right lobe of thyroid and two parathyroids of left lobe removed on May 25. For the twenty-four hours immediately following the operation there was no tetany; the calcium of the urine remained unchanged, but the magnesium was almost doubled. The increase was still apparent the next day when the animal had severe tetany from which it partially recovered only to die in a similar convulsive seizure the day following. The volume of the urine was decreased following the operation and the specific gravity was raised. There was no increase in the calcium and magnesium of the feces.

From the tabulated results and charts the following will be noted: In the brain analyses, the magnesium content is practically the same in both normal dogs' brains and those dying with tetany, but the calcium content of the brains of the two dogs dying with tetany is

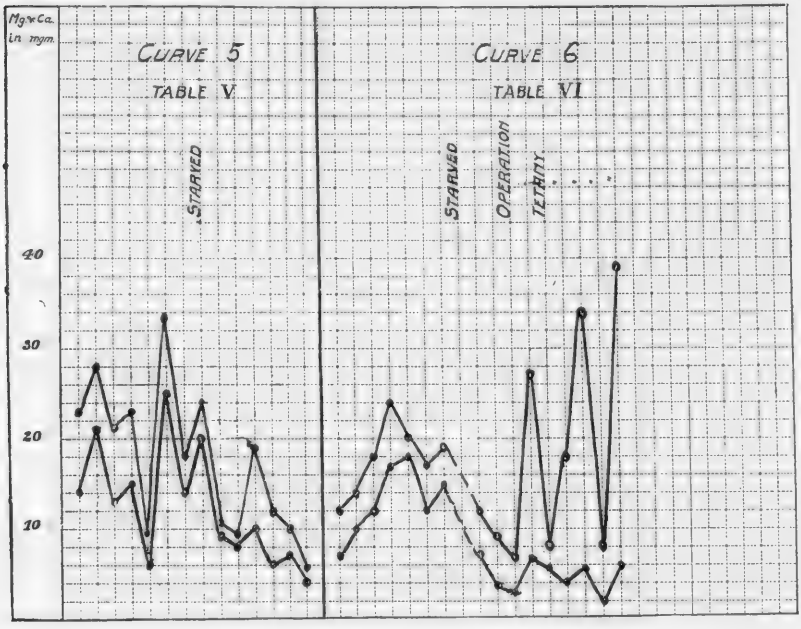
slightly higher than that of the control dogs. The excretion of calcium and magnesium in the feces of both normal and parathyroidectomized dogs shows little of interest except that during fasting the excretion is very noticeably diminished. In the urine of



Curves 1 and 2 (Dog 6). Daily urinary excretion of magnesium (above) and calcium (below) illustrating the decreased elimination during starvation.

animals, both on a constant diet and during fasting, the excretion of calcium and magnesium runs practically parallel, and both are diminished markedly and constantly during starvation, with an accompanying decrease in the volume and specific gravity of the urine. After parathyroidectomy, with the animal fasting, the urine shows an increase in the magnesium excreted, an increased specific gravity and a decreased volume. The increase in the magnesium appears before any tetany can be detected. The calcium elimination in the urine after parathyroidectomy does not differ appreciably from that of the fasting period.

For several of the days on which there was an increased elimination of calcium and magnesium in the urine after parathyroidectomy, the total ash of the urine was found to be increased from fifteen to thirty per cent. Too few estimations were made, however, for this observation to be of much value; nevertheless, it would seem to indicate that possibly an increase in other salts of the urine, as sodium and potassium, may occur. In the urine of two animals



Curves 3 and 4 (Dogs 6 and 10). Illustrating the daily urinary excretion of magnesium (above) and calcium (below) after parathyroidectomy.

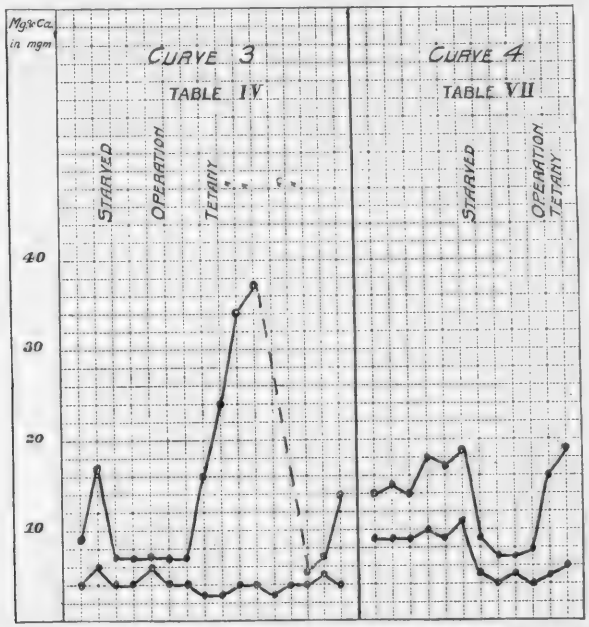
with tetany, diacetic acid was found, but a routine test was not made in all the animals and the proportion of cases showing this acid in the urine cannot be stated.

It is of interest to note in this connection the experiments of Underhill and Saiki[8] in which thyreoparathyroidectomy was performed on dogs with the development of tetany, in a certain number

[8] Underhill, F. P., and Saiki, T., Thyroid and Metabolism, *Jour. of Biol. Chem.*, 1908, v, 225.

of cases, and an increase in the ammonia nitrogen in the urine with the presence also of diacetic acid. It is probable that this increase in the ammonia, which is in accord with that found by MacCallum and Voegtlin, is due to the removal of the parathyroids.

With regard to the calcium and magnesium content of the brain,



Curves 5 and 6 (Dog 9). Curve 5 illustrates the effect of fasting; Curve 6, the effect of fasting and parathyroidectomy on the daily urinary excretion of magnesium (above) and calcium (below).

no very definite conclusions can be drawn from the small number of cases studied. The magnesium content is remarkably constant and apparently does not vary after parathyroidectomy. The fact that the brains of the two animals with tetany contained more calcium than the control animals indicates that the diminution described in the calcium content of the brains of animals with tetany is not constant.

These experiments, it may be seen, do not support MacCallum's views concerning the control of calcium metabolism by the para-

thyroids. The argument that the parathyroids control calcium metabolism, because in tetany there is a decreased calcium content of the brain and increased elimination in the urine and because tetany is relieved by injections of calcium, is not necessarily conclusive. We have evidence (Berkeley and Beebe) that other salts, such as strontium, have an effect on the tetany as striking as that of calcium; also, as here shown, that the diminished calcium content of the brain and the increased calcium content of the urine are not constant factors. The beneficial effect of venesection and saline infusion on the tetany likewise weakens this calcium control hypothesis.

One point which has probably not been sufficiently emphasized among the investigators of the parathyroid is that while the tetany is the most prominent and evident manifestation of the absence of parathyroid secretion, yet the tetany itself is but a symptom, due to an increased excitability of the central nervous system, and may in reality be of relatively minor importance. For example, reference has been made before in this paper to the fact that the increase in the calcium and magnesium in the urine after parathyroidectomy is apparent *before* the tetany can be noticed. Thus it appears that the disturbance resulting from the absence of parathyroid secretion is not only present, but must apparently reach a certain probably alarming degree before the tetany appears. In addition all investigators have noted a few unexplained cases in which, after excision of the parathyroids, the animal sinks into a listless, lethargic condition and dies without the development of a frank tetany. And finally, Berkeley and Beebe[9] (and in one instance I found the same to be true by the use of injections of calcium), have noted that the tetany may be controlled by injections of parathyroid nucleoprotein, so that the animal remains relatively free from any muscular twitchings, but, nevertheless, becomes emaciated and cachectic and finally dies, evidently from some severe nutritional disturbance. This last, especially, indicates that the animal does not die from the tetany directly, but from some less apparent, but powerful functional disturbance, although the tetany, when present, undoubtedly contributes to the fatal outcome.

[9] Berkeley, W. N., and Beebe, S. P., A Contribution to the Physiology and Chemistry of the Parathyroid Gland, *Jour. of Med. Research*, 1909, xx, 149.

It is, therefore, possible in studying the parathyroid to distinguish two fairly distinct series of phenomena; first, a metabolic disturbance resulting from the absence of parathyroid secretion, and second, the tetany which follows and is probably caused in some manner by this primary derangement. The few facts known about the physiology of the gland and the rather uncertain knowledge of the intermediary metabolism of protein, with which the parathyroid seems to be connected, make the exact nature of this disturbance rather obscure. However, after parathyroidectomy, the increased ammonia content of the blood, the increased output of nitrogen and ammonia with the increased ammonia ratio in the urine, the presence of an increase in the magnesium content and the finding of diacetic acid in some cases in the urine, and finally the good effect on the tetany of bleeding the animal, are very suggestive of an acid intoxication in connection with some disturbance in metabolism, possibly of protein. If we assume then that the parathyroid secretion is concerned in the oxidizing of certain of the intermediary products of protein catalysis, for which there is at present no evidence, and that in the absence of this secretion certain acid products accumulate which require parathyroid secretion for further oxidation, we have conditions which may explain many of the changes noted. The acid products are combined with ammonia and the bases and excreted and there is consequently an increased amount of these substances found in the urine. The loss of energy to the body by the inability to utilize these unoxidized products and the increased energy needed during the tetany require additional protein to be burned and hence the increased nitrogenous excretion in the urine. It is of interest to note that in one experiment (Dog 6) the calcium introduced intramuscularly to cure tetany was excreted not in the urine but in the feces.

SUMMARY.

1. The brains of dogs dying with tetany contain a slightly greater amount of calcium than do those of normal dogs, which would indicate that a decreased calcium content of the brain is not constant in tetany. The magnesium content is practically the same in both normal and tetany dogs.

2. The calcium and magnesium content of the feces of normal

and of parathyroidectomized dogs is similar. During fasting the excretion of both metals is diminished.

3. The excretion of magnesium in the urine in dogs on a constant diet, as well as in those fasting, runs parallel with that of calcium. The excretion of both metals is markedly diminished during fasting. After parathyroidectomy, with the animal fasting, the elimination of magnesium is greatly increased while that of calcium remains unchanged. The augmentation of the magnesium begins before tetany is observed.

4. The increased elimination of magnesium indicates that, although there is a disturbance of inorganic equilibrium, it is not limited to calcium.

It is suggested, therefore, that the tetany represents a condition of altered salt equilibrium in the nerve cells brought about by a disturbance in the catalytic processes of the body which increases the acid factors.

THE DEXTROSE CONSUMPTION BY THE ISOLATED PERFUSED HUMAN HEART.[1]

By HUGH A. STEWART, M.D.

Late Fellow of the Rockefeller Institute for Medical Research, New York.

(*From the Hunterian Laboratory, Johns Hopkins University, Baltimore.*)

That there is a consumption of the dextrose of the circulating fluid by the isolated and perfused mammalian heart was first proved by Johannes Müller (1), in 1903, and shortly after him by Locke and Rosenheim (2), in 1904. Müller, who was studying at the time the source of muscle energy, noticed in four experiments that the perfused heart of the cat caused the disappearance of a definite amount of the dextrose from the perfusion fluid. He does not state in his protocols the weights of the hearts which he studied, but if we take them to weigh eighteen grams, his experiments show an average consumption per gram weight of heart muscle per hour of 1.3 milligrams of dextrose. This result is in close accord with that obtained by Locke and Rosenheim, who worked on the rabbit's heart. These authors report an average consumption of 1.4 milligrams of dextrose per gram weight per hour. They allowed the heart to beat for seven to ten hours in an apparatus suited for a continuous circulation of fluid, and they noticed a disappearance of an amount of dextrose, proportional to the duration of the experiment. While Müller was the first to publish figures to show that the perfused heart possesses the power of consuming dextrose from the perfusion fluid, to Locke and Rosenheim is due the credit for demonstrating that there is a definite relation between the amount of sugar consumed and the length of time during which the heart is kept beating. In the same research, these authors elicited the fact that the consumption of dextrose is dependent upon a certain concentration and balance of the calcium and potassium in the perfusion fluid, and they conclude that these salts, more especially

[1] Aided by a grant from The Rockefeller Institute for Medical Research. Received for publication October 18, 1909.

calcium, are necessary for the conversion of the chemical changes into mechanical work.

Shortly after the appearance of Locke and Rosenheim's work, an Italian observer, Camis (3), published an extensive research on the dextrose consumption by the hearts of different species of animals. He took the rabbit's heart as a type of the herbivora, and in nineteen experiments he found a distinct consumption during the activity of the heart, and this consumption, per unit of mechanical work, increased with unit augmentation of load on the heart. In carnivorous animals, however, Camis's results show no breaking down of the dextrose of the perfusion fluid. In nine experiments on the cat's heart he found in only one a slight diminution of dextrose, while eight showed that the quantity of dextrose had either not changed, or had slightly increased in amount. In the heart of the fox also he was unable to detect any breaking down of carbohydrate.

These results led Camis to study the effect of the contraction during perfusion on the glycogen content of the heart. For this purpose he estimated the percentage of glycogen in the hearts of six cats which had been isolated in the usual way, but were not allowed to beat. He found the average to be 0.338 per cent., while the percentage of glycogen in nine hearts which had beat for varying periods of time was only 0.115 per cent. The loss of glycogen was presumably that which had supplied the energy for the heart's contraction. Camis considers it a characteristic of the carnivorous heart to draw upon its own resources of energy, viz., the glycogen of the heart muscle, before using up the dextrose of the perfusion fluid. In herbivorous animals, on the contrary, the isolated heart draws first on the dextrose of the perfusion fluid before using up its own store of glycogen.

It will be evident that the experimental results obtained by Camis on the dextrose consumption of the cat's heart stand in contrast to those obtained by Müller. Camis finds no breaking down, while Müller noted a disappearance of from 0.05 to 0.152 milligrams. This disagreement in their results on the cat's heart can be explained, if Camis's contention is correct, on the assumption that, as the cat is no longer a purely carnivorous animal, these observers were dealing with animals which differed in their mode of feeding.

It seemed to the writer to be of interest to determine whether the human heart possesses the power of breaking down dextrose when artificially perfused.

In dealing with the isolated human heart, it is a *sine qua non* that the heart has stopped beating before it is connected with the perfusion apparatus. A variable time must elapse between the death of the patient and the commencement of perfusion. It is conceivable that, in this interval, changes may have occurred in the heart which may disturb the metabolism which occurs when it is isolated, and perfusion is commenced before it has ceased to beat. In the case here investigated, two and one half hours elapsed between the death of the patient and the commencement of perfusion.

In order to test whether there is any material difference in the sugar consumption by a heart which has been isolated in such a manner that it has not, during isolation, ceased to beat, as compared with one which has been isolated several hours after death of the animal, two series of experiments were made. In the first series of seven experiments, the heart of the cat was isolated in such a way that the perfusion fluid was turned on through a cannula inserted into the brachiocephalic artery before the heart had ceased to beat. The perfusion apparatus which was used was that devised by Locke and Rosenheim. This consists of an ingenious arrangement whereby the oxygen pressure is utilized to make a continuous circulation of the perfusion fluid. The great advantage of this arrangement lies in the fact that the heart can be kept beating for many hours on a supply of only 200 cubic centimeters of Locke's solution. In determining the amount of dextrose in the fluid at the termination of the experiment the method of Benedict was used. The method consists in the reduction of the copper by dextrose with the subsequent formation of white cuprous sulphocyanide. The principle of this method is that a colorless, insoluble compound of copper is formed instead of the red cuprous oxide of Fehling's method. The end reaction is the disappearance of the blue color, leaving a colorless supernatant fluid and a white precipitate. In dealing with the dilute solutions which must necessarily be used in the perfusion fluids used for the heart, the writer has found it advantageous to employ a modification of their method as recommended by Benedict. A

definite measured amount of the fluid to be tested is run into a beaker containing the copper solution, the mixture boiled, and the remaining copper which has not been reduced is estimated by a one-half per cent. solution of dextrose. The results of the first series of experiments are given in the following table.

TABLE I

Date.	Duration in hours.	Amount of dextrose before perfusion.	Amount of dextrose after perfusion.	Decrease of dextrose.	Weight of heart.	Consumption of dextrose per gram weight per hour.
		Grams.	Grams.	Grams.	Grams.	Milligrams.
July 1	2	2.000	1.906	0.94	23.5	2.0
February 2	2¾	3.000	2.874	0.126	17.0	2.7
" 3	2	2.000	1.936	0.064	19.5	1.6
" 5	1¾	2.000	1.946	0.054	18.5	1.7
" 10	3½	2.000	1.904	0.096	16.2	1.7
" 11	3	2.000	1.960	0.040	12.3	1.1
" 16	3	2.000	1.919	0.081	13.0	2.1
						Average 1.8

It is evident from these experiments that heart of the cat causes the disappearance of a quantity of dextrose from the nutrient fluid which is proportional to the duration of the experiment and the weight of the heart. They show an average consumption of 1.8 milligrams of dextrose per gram weight of heart per hour.

In the second series of experiments, the animal was first killed with ether and two and a half hours later the heart was isolated and perfused with sugar-free Locke's solution, until it began to beat regularly and the perfusion fluid came through free from blood. Thereafter, a weighed amount of dextrose was added to the stock solution and circulation of the fluid commenced.

The results of these experiments are given in Table II.

TABLE II

Date.	Duration in hours.	Amount of dextrose before perfusion.	Amount of dextrose after perfusion.	Decrease of dextrose.	Weight of heart.	Consumption of dextrose per gram weight per hour.
		Grams.	Grams.	Grams.	Grams.	Milligrams.
February 20	2¼	2.000	1.932	0.068	19.0	1.6
" 24	2¼	2.000	1.936	0.064	18.3	1.4
" 16	1½	2.000	1.971	0.029	16.5	1.2
March 1	2	2.000	1.956	0.044	17.2	1.3
" 5	2	2.000	1.962	0.038	21.5	0.9
" 10	2½	2.000	1.955	0.045	18.0	1.0
" 13	1	2.000	1.985	0.015	14.5	1.1
" 18	1¾	2.000	1.958	0.042	17.5	1.6
						Average 1.3

The average consumption per gram weight of heart muscle per hour is 1.3 milligrams of dextrose, as compared with 1.8 milligrams in the first series. This slight difference is to be expected since the isolated and perfused heart is at best a slowly dying organ, and its activity both metabolic and mechanical must gradually wane the longer it is kept after the death of the animal. The comparison of the results justifies us, however, in accepting those which are to be obtained from the isolated human heart as a close approximation to normal.

PERFUSION OF THE HUMAN HEART.

The heart studied was that of a woman 46 years of age, who died in the hospital at 8 p. m. Autopsy was performed at 10 p. m. and the heart isolated. It weighed 368 grams and showed no gross pathological changes except a slight hypertrophy of the left ventricle. At 10.24 p. m. it was attached to the perfusion apparatus. The aorta was previously cut off close to the mouth of the ventricle and a glass cannula inserted into either coronary artery. The cannulas were connected by means of a rubber tubing to a Y-shaped glass tube, which in turn was attached to the reservoir containing the perfusion fluid. A sugar-free Locke's solution was turned on under pressure of 60 millimeters of mercury. The fluid was run to waste until it was free from a red tinge.

The further course of the experiment is given in the following protocol:

Time.	Rate per minute.	Temperature.	Remarks.
10.34 p.m.	80	37°	Auricles began to beat; only a visible pulsation in the ventricles.
10.39 p.m.	94	37.1°	Contractions in the auricles and ventricles gaining in strength—slight irregularity in rhythm.
10.45 p.m.	84	37.1°	Heart beating strongly. Two grams of dextrose added to stock solution and circulation of fluid begun.
10.30 p.m.	84	37°	Heart beating well.
10.59 p.m.	91	37°	Slight irregularity in rhythm.
11.05 p.m.	90	37.3°	Beats more regular.
11.15 p.m.	90	37.5°	
11.25 p.m.	88	37.5°	Strong and regular contraction.
11.40 p.m.	87	37.1°	

Time.	Rate per minute.	Temperature.	Remarks.
11.55 p.m.	84	37.2°	
12.05 a.m.	93	37.2°	
12.15 a.m.	96	37.2°	
12.30 a.m.	89	37°	Contractions not so strong.
12.45 a.m.	91	37.1°	Perfusion of sugar containing solution stopped and fluid collected.
12.48 a.m.			Sugar-free solutions turned on.
1.05 a.m.			Washings and stock solution mixed. Total amount 1,980 c.c.

The analytical figures for the estimation of dextrose in this solution are given below.

As previously stated, the method adopted was to place in a beaker 10 cubic centimeters of each of Benedict's solutions, which required for reduction 0.073 milligrams of dextrose, and 30 cubic centimeters of the sugar solution to be tested are then run in and the mixture boiled. The residual copper is estimated by titration with a 0.5 per cent. dextrose solution. For accurate results, it is necessary to precipitate the trace of albumin which is always washed out of the heart during perfusion. This was done by the addition of concentrated sodium chloride solution acidified with acetic acid.

	First sample. Amount 100 cubic centimeters.	Second sample. Amount 100 cubic centimeters.
Volume of sample after addition of NaCl solution.....	120 c.c.	120 c.c.
No. of c.c. of 0.5 per cent. dextrose solution required to reduce residual copper.		
First titration	10.8 c.c.	10.9 c.c.
Second titration	11.0 c.c.	11.3 c.c.
Third titration	10.6 c.c.	10.8 c.c.
Average	10.8 c.c.	11.0 c.c.
Residual copper.....	= 0.054 gm. dextrose	= 0.055 gm. dextrose
Copper reduced by 30 c.c. of diluted perfusion fluid.....	= 0.019 gm. dextrose	= 0.018 gm. dextrose
Amount of dextrose in 120 c.c. of diluted fluid.....	= 0.076 gm. dextrose	= 0.072 gm. dextrose
Dextrose in 1980 c.c. of undiluted fluid	= 1.505 gm. dextrose	= 1.425 gm. dextrose

Average amount of dextrose remaining in solution after 2 hours perfusion is 1.465 gm.

Consumption 0.535 gm. dextrose.

Per gram weight per hour 0.07 mg.

It is evident from this experiment that the human heart, like that of the rabbit and cat, is capable of breaking down the dextrose of the perfusion fluid. It is to be admitted that the heart here investigated is not a strictly normal one, since, as stated above, there was slight hypertrophy of the left ventricle. On that account, the figures may vary considerably from those which may be obtained from a perfectly healthy human heart. An opportunity to study a healthy heart was eagerly awaited, but none presented itself. The fact, however, that a positive result is obtained from a heart which was not ideally suited for the purpose of investigation is at least sufficient proof that the human heart when isolated and artificially perfused possesses the power of breaking down the dextrose of the perfusion fluid.

APPENDIX.

Method of Isolating the Heart.—The animal is anæsthetized with ether and bled from the common carotid artery. When blood ceases to flow, the thorax is opened by resection of the ribs near the spine so as to insure a good exposure of the thoracic organs. A ligature is slipped round the aorta above the origin of the first intercostal artery, the aorta is divided below the ligature and the left subclavian artery is ligated near the aorta. A cannula is inserted into the brachiocephalic artery and a sugar-free Locke's solution is turned on while the heart is still beating. When the heart has been washed out thoroughly, the ligature around the aorta is tightened and perfusion is thus conducted through the coronary arteries. If the operation is carried out expeditiously, there is no interruption of the heart beat. The pericardium is now opened, the superior and inferior vena cava and the pulmonary vessels divided and the heart is transferred to a moist chamber. Perfusion is carried on under a pressure of 40 mm. of mercury and a constant temperature of 37° C. To obtain a continuous circulation of fluid, the apparatus used is, with a few minor modifications, that devised by Locke and Rosenheim. After the lapse of 15 to 20 minutes, during which the heart has been thoroughly washed out of blood and the perfusion fluid comes through clear except for a slight opalescence, a weighed quantity of dextrose is added to the stock perfusion fluid so as to give approximately a concentration of from 0.1 to 0.3 per cent. Circulation of the perfusion fluid, which up to this stage has been run to waste, is established, and the heart allowed to beat for several hours. At the termination of the experiment the sugar-containing solution is collected and the heart is kept beating for 10 minutes on sugar-free solution so as to wash thoroughly the heart free of dextrose. This is added to the original perfusion fluid, as are also the washings by distilled water of the apparatus. For the isolation and perfusion of the human heart, the following method was adopted. The heart is first placed in a basin of normal saline solution at 35° C. and gently manipulated under the finger tips in order to remove all blood clot.

This operation is of considerable importance, for unless the blood clot is thoroughly removed, small particles will ultimately get into the coronary arteries and interfere seriously with perfusion. Not only has the blood in the coronary vessels themselves to be removed by gentle pressure, but it has also to be removed from the cavity of the ventricles and auricles. The latter process is apt to be tedious and unless great care is exercised it may be so damaged that it may not commence to beat when attached to the perfusion apparatus. In the preliminary experiments on the cat's heart, it was found that the most satisfactory way of removing blood from the ventricles was by way of the aorta and pulmonary artery. A fine blunt-pointed dissecting forceps is passed through the mouths of these vessels into the cavity of the ventricles, the semilunar valves are pressed aside and by the aid of reflected light the intraventricular blood clot is gently picked off. It is safer to deal with the clot in the left auricle through the opening of the inferior vena cava. It is essential that no damage be done to the sinus from which the wave of contraction takes its origin. When all blood clot has been detached and removed, the cardiac cavities are flushed with normal saline until the washings cease to have a red tinge. The heart is now attached to the perfusion apparatus. The aorta is cut close to the mouth of the ventricle and a glass cannula is inserted into each coronary artery. The cannulæ are connected by rubber tubing to the ends of a Y-shaped glass tube which in turn is attached to the reservoir containing the perfusion fluid. In dealing with the dead cat's heart, it was found that shortly after the perfusion fluid is turned on, the ventricles go into fibrillary contraction instead of a normal rhythm. This accident can as a rule be obviated by using a perfusion fluid containing a slightly higher percentage of potassium than is generally recommended.

The solution which we have found most satisfactory is one containing sodium chloride, 0.9 per cent.; calcium chloride, 0.024 per cent.; potassium chloride, 0.035 per cent.; sodium bicarbonate, 0.03 per cent.

BIBLIOGRAPHY.

1. J. Müller, Studien über die Quelle der Muskelkraft. I Mittheilung: über den Zuckerverbrauch bei der Muskelarbeit, *Zeit. f. allg. Physiol.*, 1903, iii, 282.
2. Locke and Rosenheim, Contributions to the Physiology of the Isolated Heart. The Consumption of Dextrose by Mammalian Cardiac Muscle, *Jour. of Physiol.*, 1907, xxxvi, 205.
3. M. Camis, Sur la consommation d'hydrates de carbon dans le coeur isolé functionnant, *Arch. ital. de biol.*, 1908, l, 33.

AN EXPERIMENTAL STUDY OF OPSONIC IMMUNITY TO STAPHYLOCOCCUS AUREUS.[1]

By J. C. MEAKINS, M.D.

Resident Pathologist to the Presbyterian Hospital of New York.
(From the Pathological Laboratory of the Presbyterian Hospital of New York.)

The cause of the acquired immunity to *Staphylococcus aureus* has not been definitely established. An increase of the bacteriolytic power of the serum has never been demonstrated even after prolonged immunization. Agglutinins are increased, sometimes to a very marked degree, but increase of agglutinins in many instances has little relation to increased resistance to infection. In all infections with staphylococcus there is local as well as a general leucocytosis, and it has been shown that *Staphylococcus aureus* produces a soluble thermostable substance which is strongly chemotatic for polymorphonuclear leucocytes. Besides this substance, van der Velde[2] found that *Staphylococcus aureus* produces a body called by him leucocidin, which destroys leucocytes and produces pus. All the polymorphonuclear leucocytes attracted by staphylococci to the point of infection are not destroyed, and Kirch[3] in 1889 was the first to observe that polymorphonuclear leucocytes ingest the staphylococcus. Many observers have since demonstrated that leucocytes can not only ingest but also destroy staphylococci after ingestion.

The first experiments made to explain the protective properties of staphylococcus immune serum were those of Pröscher.[4] He injected immune and normal sera into animals, and the following day introduced living staphylococci into the peritoneal cavities of these animals. Leucocytes soon accumulated, and in those animals into which the immune serum had been injected, phagocytosis was very marked, while very little phagocytosis occurred in the animals which had received normal serum. These results suggested that

[1] Received for publication September 1, 1909.

[2] *Ann. d. l'Inst Pasteur*, 1896, x, 580.

[3] Thesis, 1889, cited by Ricketts, Infection, Immunity and Serum Therapy, Chicago, 1906.

[4] Cited by Ricketts, idem.

phagocytosis played an important role in the protection afforded by such immune serum.

The cause of increased phagocytosis in the protected animal was not definitely understood until Wright and his co-workers proved that phagocytosis was promoted by the presence of opsonins. The method of Leishmann, perfected by Wright, has been used for the quantitative estimation of opsonins in the serum, but it does not demonstrate that opsonins are sufficiently increased to explain the protection found in animal experiments. Moss,[5] using this method, made an exhaustive study of opsonic immunity to *Staphylococcus aureus,* and came to the conclusion that "no high degree of opsonic immunity, such as is possible in anti-toxic and bactericidal immunity, can be produced in rabbits by ordinary inoculation procedures with the *Staphylococcus aureus.*" This has been the experience of nearly all who have worked with these methods.

In 1906, Simon, Lamar and Bispham[6] advocated a modification of the opsonic index of Wright. The opsonic power of diluted immune serum was compared with diluted normal serum of the same strength. This method undoubtedly gave a more accurate index of the opsonic power of the immune serum, but the dilutions were not carried far enough. Neufeld and Hüne,[7] and Klien[8] independently determined the opsonic power of the serum of animals immunized to *Bacillus typhosis* and allied organisms by a method of dilution which sought the point at which phagocytosis ceased altogether. By this means, a high degree of opsonic immunity was demonstrated. Marshall[9] applied a modification of this principle of dilution to the investigation of staphylococcus immunity in animals. He diluted the serum with thirty-two times its volume of fluid, and came to the conclusion that in this dilution (1 to 32) the serum causes phagocytosis with the same activity as in the undiluted state. The opsonic index of the undiluted serum, he maintained, is no guide to the manner in which a serum will behave on dilution. He did not, however, carry the dilution of the serum to the point of opsonic extinction.

[5] *Bull. of the Johns Hopkins Hosp.,* 1907, xviii, 237.
[6] *Jour. of Exper. Med.,* 1906, viii, 651.
[7] *Arb. a. d. k. Gsndhtsamte,* 1907, xxv, 164.
[8] *Bull. of the Johns Hopkins Hosp.,* 1907, xviii, 245.
[9] *Jour. of Bact. and Path.,* 1908, xii, 378.

I have demonstrated by the method of dilution that an increase of opsonic activity comparable to the increase of bactericidal, antitoxic and agglutinating powers may be produced in animals immunized with the following living bacteria: *Bacillus dysenteriæ, Bacillus tuberculosis, Streptococcus pyogenes,* and in a less degree with *Staphylococcus aureus.* In rabbits immunized to *Staphylococcus aureus,* abscess formation always occurred, but when a few doses of staphylococcus vaccine were first administered, the animals resisted small amounts of the living organism much better than those animals to which no vaccines had been given. This fact confirmed previous observations which showed that killed cultures of staphylococcus could produce a certain amount of immunity.

The following experiments have been undertaken to determine what degree of immunity can be produced by the administration of killed cultures or vaccines, and to determine whether such an immunity has a definite relation to the opsonic activity of the serum. Since it has never been possible to demonstrate any bactericidal properties in staphylococcus immune serum, the experiments were confined to the estimation of the opsonic power of the serum.

Methods.—The technique used was similar to that employed in my previous experiments, which is essentially the same as that devised by Klien. The staphylococcus emulsion, leucocytic emulsion, and blood serum were collected by the methods of Wright. The serum, however, was diluted with Ringer's solution. Specimens were prepared from each dilution in the pipettes used by Wright and after incubation at 37° C. for fifteen minutes smears were prepared from each pipette. A specimen in which Ringer's solution was substituted for the diluted serum was prepared with each set of experiments. The smears were stained with Hasting's modification of the Romanowski stain, and the number of cocci in fifty leucocytes was counted for each specimen. A large number of observations made with normal rabbit serum demonstrated that the opsonic power of normal serum had almost constantly disappeared after a dilution of one to thirty. Such extinction of phagocytosis by dilution was so constant that it was not considered necessary to make normal control dilutions with normal serum for comparison with each experiment (see Chart I). The strain of

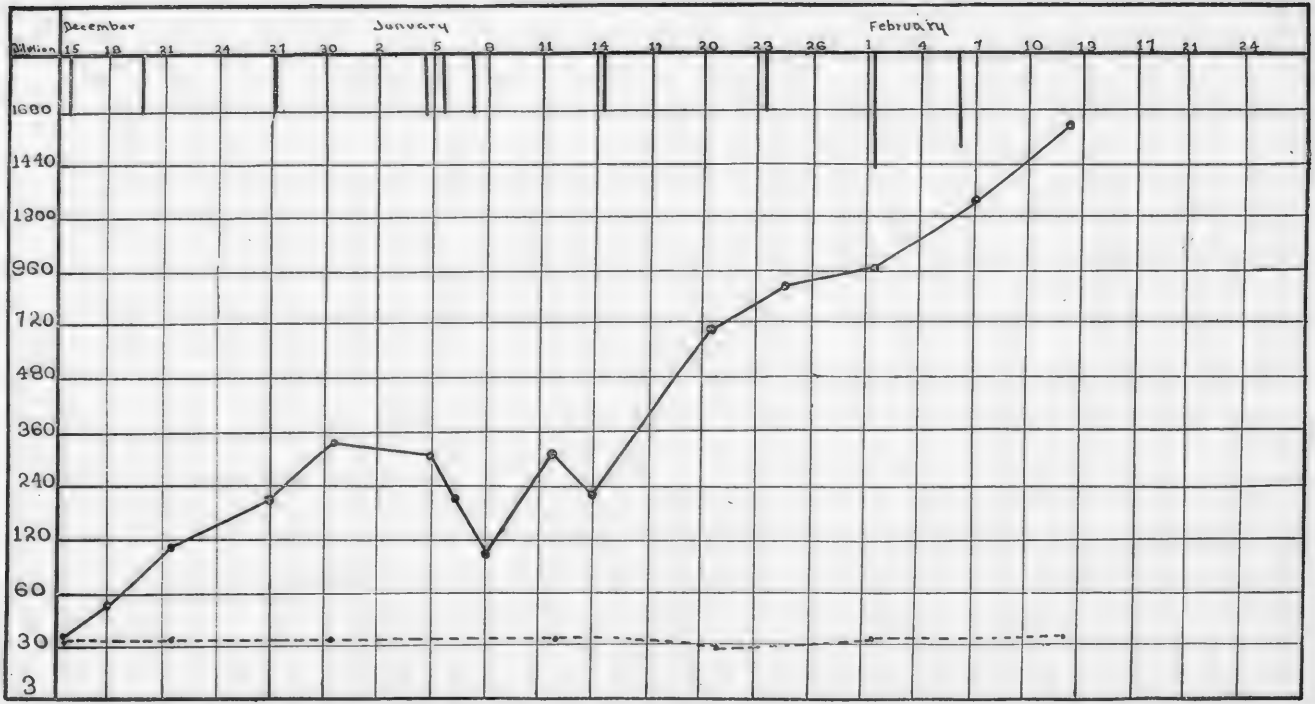


CHART. I. Heavy straight line represents opsonic curve of immunized rabbit; dotted line represents opsonins of normal rabbit.

Staphylococcus aureus used for the opsonic determinations and for preparation of the vaccines was obtained from the blood of a patient with severe pyemia. It was very virulent for rabbits and underwent phagocytosis of only moderate activity. The leucocytes were obtained from normal human blood drawn into a solution of sodium citrate, and washed three times with Ringer's solution. The vaccines were prepared from a twenty-four hour old culture, grown on plain agar. The organisms were killed by heat at 60° C. for one-half hour. One-half cubic centimeter of the vaccine was introduced into a flask of bouillon; the vaccines tested by this method were uniformly sterile. The number of organisms per cubic centimeter was not counted, but the dosage of the vaccine was determined by inoculating the growth from a certain number of agar slants.

EXPERIMENT I.—A rabbit weighing 1,950 grams was used. The opsonic power of the serum determined several times before the first vaccination was found to disappear between a dilution of 1 to 30 and 1 to 45. On December 5, 1908, a vaccine of *Staphylococcus aureus* was prepared from the growth on two agar-agar slants, and injected subcutaneously (all subsequent vaccinations were made by this method). The vaccinations were repeated at intervals of from seven to ten days (see Table I), and the opsonic power of the serum was determined at intervals of from three to five days (see Chart I).

TABLE I.

No.	Date of vaccination.	Amount of vaccination.
1	December 15, 1908.	2 agar-agar slants.
2	December 19, 1908.	2 agar-agar slants.
3	December 27, 1908.	2 agar-agar slants.
4	January 3, 1909.	2 agar-agar slants.
5	January 5, 1909.	2 agar-agar slants.
6	January 7, 1909.	2 agar-agar slants.
7	January 15, 1909.	2 agar-agar slants.
8	January 24, 1909.	2 agar-agar slants.
9	February 2, 1909.	3 agar-agar slants.
10	February 8, 1909.	2½ agar-agar slants.

The opsonic power gradually increased and after sixty days the dilution in which phagocytosis ceased was one to fifteen hundred, or practically fifty times the opsonic power of the serum before the vaccination. The opsonic power of a normal rabbit was frequently determined for control of the vaccinated animal and was found to vary little from day to day. The curve representing the opsonic activity of the serum of the vaccinated animal diverged from the curve representing the normal animal and the increase of opsonins was progressive with only one unexplainable remission, which might have been due to an error of technique.

In order to determine whether a negative phase of opsonic activity occurred after inoculation, vaccines were given at close intervals. On January 3, 5 and 7, respectively, vaccine prepared from the growth on two agar-agar slants was given and the opsonic power was estimated every second day. There was found to be a diminution in the amount of opsonins present in the serum and this diminution increased after each successive dose, but quickly disappeared when the period between the vaccinations was prolonged. This rabbit received altogether as vaccines the growth from twenty-one and a half agar-agar slants, divided into ten doses. At the site of each injection a small mass appeared after a few days. The characters of these masses will be described later. There was a slight increase of weight during the period of vaccination and the animal continued in excellent condition. On February 14, however, it was found moribund, and died during the day. An autopsy was performed one hour post-mortem. The liver was very large, of a blackish color and filled with coccidial cysts. Microscopical examination showed that the liver contained a large amount of black pigment. The other viscera were normal. Cultures from the liver, spleen, kidneys, lungs and heart's blood remained sterile. The cause of death was not determined.

Since this experiment had demonstrated that the serum develops a high opsonic power for *Staphylococcus aureus* when rabbits immunized by vaccines prepared from this microörganism were used, further experiments were undertaken to confirm this finding, and to determine, if possible, what protection such increase of opsonins might afford.

Six rabbits were vaccinated as in the first experiment, and the opsonic power estimated at irregular intervals in order to follow the progress of the immunity. The vaccines were injected subcutaneously every seven days. At the point of each inoculation a small indurated lump developed. A few of these lumps were incised and thick white pus evacuated; it was always sterile. This abscess formation was evidently due to the power of the dead staphylococcus to cause a local accumulation of leucocytes. The majority of the swellings produced by the vaccines were not opened and were gradually absorbed; in no case did spontaneous rupture occur.

The opsonic power gradually increased in a manner similar to that recorded in Experiment I, but Table II shows that the production of opsonins did not occur with equal rapidity in all of the animals. Rabbit 2, and especially Rabbit 5, acquired an increased opsonic power very quickly. Rabbit 3 produced opsonins very slowly.

TABLE II.

Number.	Weight of rabbits.	Number of vaccinations.	Total amount of vaccines.	Time under treatment.	Opsonic power before vaccination.	Opsonic power after 20 days.	Opsonic power after 45 days.	Opsonic power after 70 days.	Opsonic power after 95 days.	Opsonic power after 121 days.
1	1690 gm.	15	25 agar slants.	121 days.	1 to 45	1 to 120	1 to 480	1 to 960	1 to 1200	1 to 1500
2	1650 "	15	25 " "	121 "	1 to 45	1 to 150	1 to 720	1 to 1200	1 to 1500	1 to 1800
3	1680 "	11	19 " "	95 "	1 to 30	1 to 120	1 to 360	1 to 720	1 to 960	—
4	1950 "	11	19 " "	95 "	1 to 30	1 to 120	1 to 240	1 to 960	1 to 1200	—
5	1720 "	11	19 " "	95 "	1 to 45	1 to 210	1 to 720	1 to 1200	1 to 1800	—
6	1700 "	11	10 " "	32 "	1 to 45	1 to 150	—	—	—	—

Rabbit 6 died suddenly after thirty-two days of immunization, having attained an opsonic power of 1 to 120. An autopsy was immediately performed and the liver exhibited a condition similar to that noted in Experiment I. There was extensive coccidiosis; the organ was dark red and microscopical section revealed slight pigmentation of the cells. Cultures from the blood, liver, spleen and peritoneum were sterile.

When the serum of Rabbits 1, 2, 3, 4 and 5 had developed considerable opsonic activity, their resistance to virulent living staphylococci was tested. The staphylococcus used was that from which the vaccines had been prepared. The virulence of this strain was increased by passing it through three rabbits. In order to determine the natural resistance of rabbits to this strain of *Staphylococcus aureus,* various quantities were injected into the ear vein of nine normal rabbits. The staphylococcus was grown on plain agar-agar and the growth from each tube was gently removed in two cubic centimeters of normal salt solution. The suspension was well shaken with glass beads to break up the clumps and prevent emboli formed by masses of bacteria.

TABLE III.

Number.	Weight.	Opsonic power.	Date of injection.	Amount of injection.	Results.
1	1,490 gm.	1 to 45	June 25	1/8 agar slant.	Died after 5 days.
2	1,600 "	1 to 45	" "	1/4 " "	" " 36 hours.
3	1,500 "	1 to 45	" "	1/3 " "	" " 24 "
4	1,535 "	1 to 30	" "	1/2 " "	" " 12 "
5	1,450 "	1 to 45	July 1	1/4 " "	" " 36 "
6	1,800 "	1 to 45	" "	1/4 " "	" " 36 "
7	1,600 "	1 to 30	" "	1/4 " "	" " 24 "
8	1,650 "	1 to 30	" "	1/4 " "	" " 24 "
9	1,200 "	1 to 30	" "	1/4 " "	" " 24 "

The results of inoculation are shown in Table III.

AUTOPSY FINDINGS IN THE CONTROL RABBITS.

Rabbit 1.—There was cloudy swelling of all viscera, with numerous abscesses in the kidneys. Cultures showed staphylococci in the blood.

Rabbit 2.—The findings were similar to those of Rabbit 1, except that abscesses in the kidneys were much smaller.

Rabbits 3 and 4.—There was marked cloudy swelling, but no abscesses were found. Cultures from all the viscera and the blood showed a pure growth of *Staphylococcus aureus.*

Rabbit 5.—Findings were similar to those of Rabbit 1.

Rabbit 6.—Findings were similar to those of Rabbit 2.

Rabbits 7, 8 and 9.—Findings were similar to those of Rabbits 3 and 4.

The immunized rabbits were injected intravenously with the same strain of *Staphylococcus aureus,* five control rabbits (see Table III) being given the same amount of bacterial emulsion on the same day. The results of inoculation of the immunized rabbits are given in Table IV.

TABLE IV.

No.	Weight.	Opsonic power.	Date of inoculation.	Amount of inoculation.	Result.
1	1,690 gm.	1 to 1,500	July 1.	¼ of agar slant.	Died after 21 days.
2	1,650 "	1 to 1,800	" 1.	" " "	Etherized after 45 days.
3	1,680 "	1 to 960	" 1.	" " "	Died after 5 days.
4	1,950 "	1 to 1,200	" 1.	" " "	Died after 13 days.
5	1,720 "	1 to 1,800	" 1.	" " "	Etherized after 45 days.

PROTOCOLS OF IMMUNIZED RABBITS.

Rabbit 1.—The animal, inoculated on July 1 with one-quarter of an agar-agar slant of *Staphylococcus aureus,* was very ill until July 8, but from this date there was slight improvement until July 12. The animal gradually lost weight and died on July 22, twenty-one days after inoculation. At autopsy the animal was much emaciated, and the skeletal muscles had a pale, cloudy appearance. The liver was very dark in color. The spleen was not enlarged. The kidneys contained a few small white areas, some of which were rather indistinct and depressed below the surface, and, on section, appeared to be scar tissue. Other white areas were even with the surface of the cortex and contained a small bead of yellowish pus. Smears from this pus showed polynuclear leucocytes and a few cocci. The pelves, ureters and urinary bladder were congested.

No growth was obtained from the heart's blood, lungs, liver, spleen and scar-like areas in the kidneys. Cultures from the small abscesses of the kidneys and from the urine contained a pure culture of *Staphylococcus aureus.*

Rabbit 2.—The animal, inoculated on July 1 with one quarter of the growth from an agar slant, gradually lost weight for two weeks following the in-

oculation, being reduced to 1,400 gm., but after July 16 it began to regain its weight, and on August 14 weighed 1,580 gm. On August 14 it was killed with ether. The liver was of a blackish color and was somewhat smaller than normal. The kidneys were pale, and in the cortex there were several opaque depressions to which the capsule was adherent.

No bacterial growth was obtained from the heart's blood, lungs, liver, kidneys and urine.

Rabbit 3.—The animal, inoculated on July 1 with the growth from one-quarter of an agar slant, was very sick the next day and died five days after inoculation. The viscera were congested and showed marked cloudy swelling. The liver, as in the other experiments, was very dark. The kidneys contained a few minute white areas, which, on section, contained a drop of yellowish fluid; smears of this pus showed pus cells and cocci.

Cultures from the heart's blood, lungs, liver, spleen, kidneys and urine contained a pure growth of *Staphylococcus aureus.*

Rabbit 4.—Inoculated as in the preceding experiments, the animal exhibited no signs of acute illness, but gradually lost weight and strength and died thirteen days after inoculation. The findings at autopsy were similar to those of Rabbit 1. No growth was obtained, except from the kidney abscesses and the urine, which contained *Staphylococcus aureus.*

Rabbit 5.—Inoculated as in other experiments, the animal, like Rabbit 2, lost weight (from 1,720 to 1,300 gm.), but at the time of etherization it had regained 350 gm. (weighing 1,650 gm.). The autopsy findings were the same as those of Rabbit 2. No bacterial growth was obtained.

The inoculations of living staphylococci into normal rabbit, especially when one-quarter of the growth from one agar-agar slant culture was employed, presented several noteworthy features. Six animals were inoculatd with this quantity; all of them died within thirty-six hours after the time of injection, three living thirty-six hours, and three, twenty-four hours. The three that lived thirty-six hours had an opsonic power of 1 to 45, whereas those living twenty-four hours had an opsonic power of only 1 to 30. The regularity of this relationship between the degree of the opsonic power and the effect of the inoculation is significant in view of the results obtained with immunized animals.

The experiments with immunized animals demonstrate what a high degree of opsonic power may be produced in rabbits by vaccination with *Staphylococcus aureus.* The dosage of the vaccines was large, as it was believed that the organisms might be subject to such change during the process of sterilization that their effect in the production of immune bodies would be diminished. The vaccines were prepared in large amounts, and the dosage was graduated

as carefully as possible. In spite of this care, the amount of opsonins produced varied greatly in different animals. Variation was probably due to individual peculiarities of the animals as they had about the same weight and age and were kept under similar conditions.

Although the immunized animals received, as nearly as could be determined, the same amount of living bacterial emulsion, the results varied greatly. One rabbit lived five days; a second, thirteen days; a third, twenty-one days; and two apparently recovered completely. Such differences seemed due to the differences in the degree of immunity in different animals. The rabbit which had the lowest opsonic power died in five days, and those with the highest opsonic power recovered (see Table IV). This observation accords with the results obtained with normal rabbits, for those with the higher opsonic power lived the longer.

Since there appears to be a direct relationship between the quantity of opsonins in the blood serum and the resistance of immune and normal rabbits to virulent *Staphylococcus aureus,* the opsonic power of the serum may afford a valuable means of determining the presence and the degree of such resistance. As bactericidal bodies have not been demonstrated for *Staphylococcus aureus,* and as agglutinins play but a small part in the destruction of bacteria, it is fair to suppose that opsonins play an important part in the cure of infection with staphylococcus.

TABLE V.

No.	Source of Staphylococci.	Opsonic power of Rabbit 1.	Opsonic power of Rabbit 2.	Opsonic power of Rabbit 3.	Opsonic power of Rabbit 4.	Opsonic power of Rabbit 5.	Opsonic power of normal rabbit.
1	Pyemia (organism used for vaccination).	1 to 1,200	1 to 1,500	1 to 840	1 to 960	1 to 1,500	1 to 30
2	Abscess.	1 to 960	1 to 1,200	1 to 720	1 to 720	1 to 1,200	1 to 30
3	Arthritis.	1 to 960	1 to 1,200	1 to 720	1 to 720	1 to 1,200	1 to 30
4	Acne.	1 to 960	1 to 1,200	1 to 720	1 to 720	1 to 1,200	1 to 30
5	Cellulitis.	1 to 960	1 to 1,200	1 to 720	1 to 720	1 to 1,200	1 to 30

Do opsonins produced by vaccination with a single strain of *Staphylococcus aureus* render other strains susceptible to phagocytosis? With this question in view, the following experiment was performed. Five cultures of *Staphylococcus aureus,* obtained from different sources, were tested with the serum of each of the five immunized rabbits. The results are given in Table V.

This experiment shows that opsonins produced by vaccination with one strain of *Staphylococcus aureus* are very powerful in promoting phagocytosis of heterologous strains, but have a somewhat greater power against the homologous strain. This observation has an important bearing upon the administration of vaccines in the treatment of staphylococcus infections of the human body. It indicates the advisability of using homologous strains of staphylococcus as vaccines in order to obtain the best results, but vaccines from other strains of *Staphylococcus aureus* might be efficacious.

Since the opsonic power of the serum plays an important part in the protection of animals infected with *Staphylococcus aureus,* it is important to ascertain what degree of opsonic immunity is developed in man during the course of infection with staphylococcus. For this purpose the following cases were investigated:

Case I.—W. C., age 37, white, single, was admitted to the service of Dr. Eliot in the Presbyterian Hospital, on March 7, 1909; he has always been in excellent health, but for many years has had to urinate several times each night. He denies any venereal infection. On the morning of March 5, 1909, while at work, the patient was seized suddenly with pain in the right flank, radiating to the right hypogastrium and the right iliac region. Until entrance into the Hospital at midnight of March 6, 1909, the pain was continuous, and was only partially relieved by anodynes. There were no urinary symptoms.

Physical examination on entrance to the Hospital showed that the patient was a large, well nourished man. The abdomen was moderately distended and rigid, particularly on the right side; there was acute tenderness, moderate fulness, and increased dulness of the right flank. The white blood cells numbered 13,800, consisting of polymorphonuclear leucocytes, 76.5 per cent., transitionals 5.5 per cent., large mononuclears 1 per cent., lymphocytes 6.5 per cent., eosinophiles 0.5 per cent. The temperature was 101.5°, pulse 102, respiration 28.

An incision was made over the right kidney along the outer border of the quadratus lumborum muscle. The tissues surrounding the kidney were infiltrated with sero-purulent fluid, and the perinephritic fat appeared necrotic. In the space between the capsule of the kidney and the cortex there was a quantity of clotted blood. The kidney itself was only slightly enlarged and much congested. The cortex was studded with small whitish areas, filled with pus, the largest being the size of a pea. The kidney was removed. A culture at the time of operation showed a pure growth of *Staphylococcus aureus.*

Microscopical examination shows an increase of the interstitial tissue; the arteries are thickened, the tubules are compressed, and the epithelium is desquamated in many places. The whole organ is riddled with miliary abscesses. The wall of the pelvis is much thickened by granulation tissue, the mucosa being ulcerated and covered with a necrotic slough.

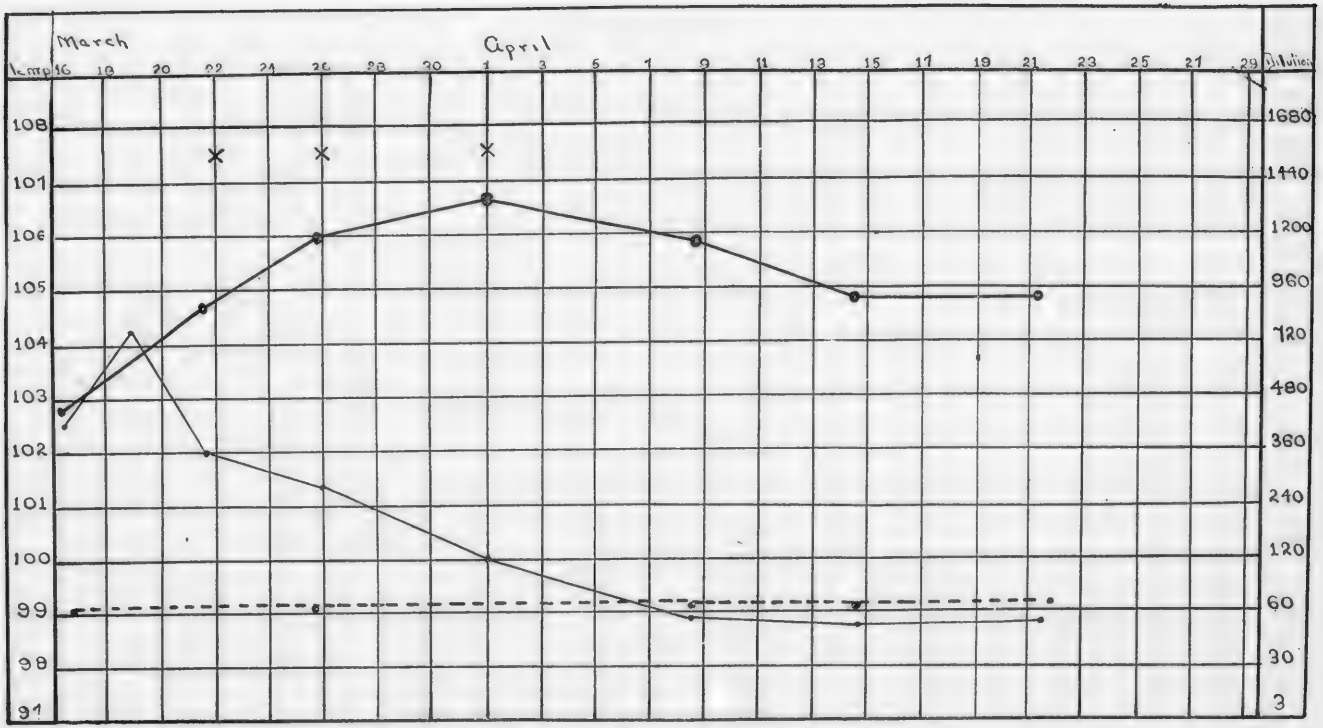


CHART II. Heavy straight line represents opsonic curve of patient; dotted line, opsonic curve of normal individual; fine straight line, temperature curve of patient; *X* represents dates of vaccination.

March 10.—The patient has been prostrated and delirious at times. He has had incontinence of urine. The temperature has gradually risen to 103.5° F., and the pulse to 120. The urine after operation was acid (sp. gr., 1,020) and contained a trace of albumin. There were hyaline and granular casts, with many pus cells and a few red blood cells.

March 18.—The wound has completely broken down, and brawny induration extends from the costal margin to the crest of the ilium. The urine has been voided in good amount, averaging 50 to 80 ounces in twenty-four hours. On this date, there is swelling of the left shoulder and axillary region, the arm, the forearm and the hand, with much tenderness along the course of the axillary vein. A blood culture made on March 14 contained a pure growth of *Staphylococcus aureus* and a culture from the urine on this date contained *Streptococcus* and *Bacillus coli communis.*

A vaccine was prepared from the *Staphylococcus aureus* found in the blood and the first dose of one hundred million cocci administered on March 22. This dose was repeated on March 26 and April 1.

April 1.—The temperature is lower and the patient's condition has improved.

April 23.—The patient continues to improve, and the wound has healed completely. The signs of phlebitis in the left arm have disappeared. The urine contains only a few casts and a trace of albumin, and is voided in good amount.

The opsonic activity of the serum was followed from March 16 until the patient left the hospital (see Chart II). There was a steady increase during the time of infection and a slight diminution during convalescence. The highest point attained was 1 to 1280, and when the patient passed from observation the opsonic power ceased at a dilution of 1 to 960. Whether the administration of the vaccine tended to increase the opsonic power is uncertain. Before the vaccines were given, the opsonic power of the serum was greater than normal; there was further increase and synchronous with this increase there was marked and continuous improvement in the patient's condition.

Case II.—M. J., age 26, male, white, had suffered for five years with repeated attacks of furunculosis, which had resisted systemic and local treatment. On April 24, 1909, a furuncle appeared on the back of the neck. It was incised but remained as a brawny induration about 3 cm. in diameter, and discharged very little pus. On April 28, a second boil appeared about 3 cm. from the first and quickly attained a large size. Merging with the first, it formed a large indurated mass about 10 cm. in diameter. Both furuncles were again incised, but incision did not have a favorable result; the induration remained and the whole of the back of the neck, on the right side, was dark red, indurated and exquisitely tender. The cervical glands were enlarged. A culture from the first furuncle contained *Staphylococcus aureus* and from this culture a vaccine was prepared. Five hundred million cocci were injected

subcutaneously, May 1, at 9 p. m. The next afternoon there was a moderate reaction at the point of injection, which increased towards evening; at this time the induration and tenderness of the furuncles had increased and the patient felt sick. On the morning of May 3, there was a marked change; the induration of the furuncles had almost disappeared, the redness and tenderness were much less, and there was a very profuse discharge of pus. Within twenty-four hours the discharge ceased, and all that remained to indicate the points of incision were two small openings. The patient's general condition was excellent, and he has remained totally free from subsequent infections during a period which has been the longest for five years.

The opsonic power of the serum was estimated several times before and after the vaccinations (see Chart III). The opsonic

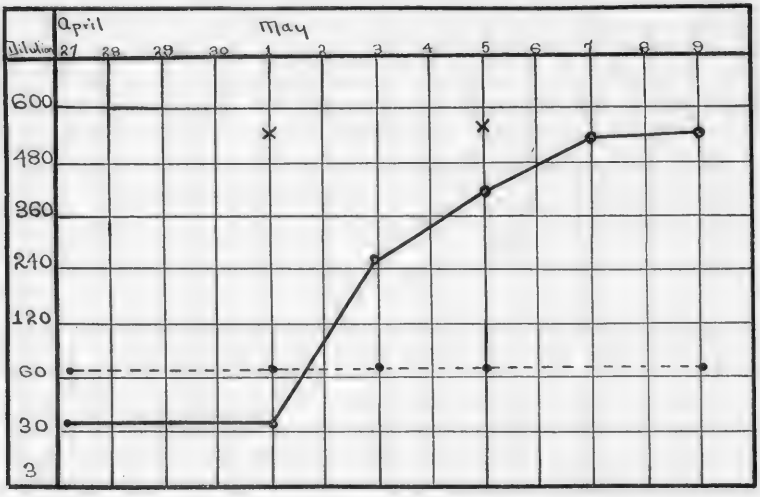


CHART III. Heavy line represents opsonic curve of patient; dotted line, opsonins of normal individual; *X*, dates of vaccination.

activity of the serum before vaccination was subnormal (1 to 30), but immediately after the first administration of vaccine the opsonic activity increased and continued to increase steadily after the second vaccination. In this case, the increase of opsonins and the synchronous improvement of the local lesion and the general condition of the patient are strong evidences in favor of the view that increase of opsonic immunity served to combat infection with staphylococcus.

CONCLUSIONS.

1. The administration of *Staphylococcus aureus,* killed by heat (vaccine), produces a high degree of opsonic immunity in rabbits.

2. Such increase of opsonin affords protection against living virulent staphylococcus in direct proportion to the amount of opsonins present in the serum and complete recovery may follow subsequent inoculation, if the opsonic power be high.

3. Frequent administration of vaccines may produce a diminution of the opsonic power of the serum.

4. Immune opsonins are most active against the homologous strain of *Staphylococcus aureus,* but are only slightly less active against heterologous strains.

5. Infections of the human body by *Staphylococcus aureus* may cause great increase of opsonins.

6. Vaccines prepared from *Staphylococcus aureus* may produce a high degree of opsonic immunity in man.

TUBERCULOUS CERVICAL ADENITIS: A STUDY OF THE TUBERCLE BACILLI CULTIVATED FROM FIFTEEN CONSECUTIVE CASES.[1]

By PAUL A. LEWIS, M.D.

(*From the Laboratory of Comparative Pathology, Harvard Medical School.*)

The study I wish to report was begun on February 1, 1907. It was thought at that time by Dr. Theobald Smith that there was adequate recognition among workers in this special field of the definite distinction between tubercle bacilli of the human and bovine type, and of the proposition that the bacilli cultivated from a certain small number of cases of human tuberculosis were typically bovine in character; that presumably the human being had become infected from the bovine in these cases and in no others. In the work done up to that time, effort had been directed toward the discovery of instances of this type of infection. It had been shown that these relatively rare cases were never cases of chronic pulmonary tuberculosis; that they were usually cases of infection by way of the intestinal tract in children, particularly cases of mesenteric lymph node disease. Several cultivations from the cervical lymph nodes had likewise given bacilli of bovine type.

It was felt that the time had come when it was possible and advisable to attempt to get definite statistical evidence as to the frequency of the infection of human beings with the bovine type of tubercle bacillus, that the technique had been developed to such a point that the work was feasible, and that the most profitable point of immediate attack would be in the field of cervical lymphatic gland infection—an important, rather frequent disease in any community, one which had been little studied from this point of view, and a field in which a certain percentage of bovine infections might confidently be expected to occur.

Accordingly, at the suggestion of Dr. Smith and under his imme-

[1] Preliminary report read at the International Congress on Tuberculosis, Washington, 1908. Received for publication, August 5, 1909.

diate supervision, I undertook to study a consecutive series of cases of tuberculous cervical adenitis with the aim of determining the type of bacillus active in each case. My sincere thanks are offered to Professor Smith for the initial suggestion and for the unceasing advice and attention which alone have made it possible for me, a beginner in this difficult field, to complete the examination of a considerable, consecutive series of cases.

LITERATURE.

There has been so much expert discussion of the relationship between bovine and human tuberculosis that any review of the general literature of the subject may properly be omitted here and I shall confine myself to a summary of those reports of cases of primary cervical adenitis in which the type of the infecting bacillus has been definitely determined.

Smith (1) has described 3 cultures of bovine isolated from tonsils, in which cases the cervical lymph nodes were infected as well. In the general series of cases from the German Imperial Health Office (2), there are reported 4 cases of primary cervical adenitis, 2 of which were due to bacilli of bovine type. At the time these cases were described, the investigators were working with the purpose of discovering bacilli of bovine type in human disease and there was more or less selection of cases. There are a number of other cases reported in which the isolations were made from cervical glands but in which there was generalized tuberculosis. Whether the cervical lymph node disease was primary, secondary, or independent in relation to the general process can not be determined from the reports and they are omitted from the present summary.

The report of the English Commission (3) shows that nine of their cultures were derived from cases of primary cervical adenitis. Of these, 3 were of bovine type.

Oehlecker (4), on the part of the German Imperial Health Office, has reported on isolations from 14 consecutive cases of primary cervical adenitis; 2 infections in adults were both due to bacilli of human type. Of 12 cases in children, 4 were due to bacilli of bovine type.

Duval (5) studied the bacilli isolated from 4 cases of primary cervical adenitis which ran an unusual clinical course. Of these cultures, 2 were of human type. A third was, in most of its features, of human type but gave the reaction of glycerine bouillon characteristic of the bovine type. In regard to the fourth culture (No. 1) the author was in doubt, but finally regarded it as probably an avian bacillus more or less modified.

Park (6) in a preliminary report gave an account of sixteen isolations from primary cervical adenitis. Two of the cultures were of bovine type.

METHODS.

The material for this study has come to my hand without selection, through the kindness of the staff and house physicians of the Children's Hospital, Boston, and the Boston City Hospital. It has been removed at operation without exception. As a rule, I have received it in moist gauze from the operating room, but in no case have I been able to control its manipulation absolutely, and I am obliged to assume that there has been no contamination with other tuberculous material before it reached my hands.

As soon as possible after receiving the material, guinea-pigs were inoculated. After three to five weeks cultures were made from the guinea-pig. First cultures were always made on Dorset's egg medium. These were uniformly successful. In each case in which the guinea-pigs became tuberculous I have obtained cultures on the egg-medium. Sub-cultures were made at intervals of from three to six weeks to parallel series of egg-medium and glycerine agar. After the glycerine agar culture has become thoroughly established in any case, I have discontinued the series on the egg-medium. The glycerine-agar culture, whenever it has been possible to obtain it, has been used as the stock culture, subsequent transfers and most inoculations having been made from this medium. In two instances, however, no growth was obtainable on glycerine agar and the egg-medium was still in use when the cultures were lost nearly two years after their isolation. Dog serum has not been used as routine, because of the difficulty in obtaining a regular supply. Consequently, the differences in morphology which characterize the two strains when grown on this medium have not been developed.

After an active growth had been established with any culture it was tested for the character of its growth on glycerine bouillon and the changes in reaction which it produced. This was done by inoculating flasks of 5 per cent. glycerine bouillon of determined acidity, usually about .2 per cent., with the culture and removing portions of the fluid at different stages of growth. These portions were titrated. The indicator used was phenolphthalein and the neutralization was done at the boiling point.

The virulence for rabbits was determined and three of the cultures were tested as to their virulence for calves. In the other cases

it has been assumed that the virulence for rabbits was a satisfactory indication of the virulence for cattle. In testing the virulence for rabbits, glycerine-agar cultures were used, as a rule. In the exceptional instances (07.11, 07.93), where no glycerine-agar culture could be obtained, cultures on egg-medium were used. Cultures from two to three weeks old were chosen, a portion of the growth was removed to a sterile test tube and rubbed up thoroughly with a glass rod. Then a little normal salt solution was added and the rubbing continued till a thick emulsion, as smooth as possible, was made. This was then diluted by the further addition of normal salt solution until it had as nearly as possible the density of a twenty-four hour culture of *B. typhosus* in plain bouillon. This suspension is spoken of through the notes and protocols as a standard suspension. This suspension is roughly equivalent to .001 gram of moist culture per cubic centimeter.

In testing the virulence of the cultures for calves, glycerine agar cultures were likewise used. The growth removed was weighed and the suspension prepared on that basis.

DETAILED STUDY OF CASES.

I have thus examined 17 cases of primary cervical adenitis. In 2 cases the guinea-pigs failed to develop tuberculosis, although microscopical examination of the tissues showed the pathological picture of tuberculosis, in one case most typically. The 15 cases in which tuberculosis developed in the guinea-pig all gave positive cultures. The protocols of these cases are given in detail in the following pages.

CASE 07.8.—The original cultures on egg-medium grew well, although not luxuriantly. The first subcultures made to glycerine agar grew, although feebly. The third subculture on glycerine agar grew fairly vigorously and was approximately as strong as a fresh culture of human type. The earliest attempts to transfer from the first and second glycerine-agar cultures to glycerine bouillon failed. A flask inoculated Jan. 30, '08, grew well in this medium. The character of the growth on this flask corresponded to that obtained subsequently on a number of occasions. The inoculation spot grew slowly to a white opaque wrinkled island 1 to 2 cm. in diam. From this, after 2 to 4 weeks, there spread out rather suddenly a thin veil-like membrane which covered the surface of the medium in from 1 to 2 weeks more. At the maximum of growth, the thicker spots had become warty in appearance. The remainder of the membrane was relatively thin and translucent with distinctly heavier white opaque veining. The membranes showed little tendency to fall.

Reaction on Glycerine Bouillon.—3 different lots of bouillon were inoculated. The initial acidity in 2 cases was 2 %. In one case, 1.5 %. In 4 of the 5 flasks inoculated, the acidity, at the time when the surface was just covered by membrane, had fallen to from .4 % to .7 %. The first flask of the series (Jan., '08) was not again opened for 3 months. The acidity at this time was 3 %. The membrane was macroscopically normal and no microscopic examination was made. A second flask (July, '08) became contaminated with mould. In Nov., '08, 3 flasks of the same lot of bouillon were inoculated. The depth of the medium was varied to give a thin, medium, and thick layer. One of these grew very slowly, the acidity slowly fell from 1.5 % to 1 % in 3½ months. In 2 others, the growth was normal. Surface covered in 5 to 7 weeks. Reactions 0.4 % and 0.7 % acid at this time. From this point the acidity gradually increased so that it was finally in one case 1.5 % (7 weeks after surface was covered), and in the other case, 1.3 % (5 weeks after surface was covered). Cultures macroscopically and microscopically pure. The animal tests gave the following result:

Animal.	Weight.	Date.	Dose.	Injection site.	Result.
Rabbit 259	2360 gm.	June 7, '07	½ c.c. stand. susp.	Intravenous.	d. 19 days. Generalized tuberculosis.
Rabbit 330	2520 gm.	April 3, '08	½ c.c. " "	Intravenous.	d. 19 days. Generalized tuberculosis.
Rabbit 338	2230 gm.	June 18, '08	$\frac{1}{200}$ c.c. " "	Intravenous.	d. 32 days. Generalized tuberculosis.
Calf 201	230 lb.	Aug. 12, '08	5 mg.	Intravenous.	d. 17 days. Generalized tuberculosis.
Calf 203	175 lb.	Aug. 12, '08	5 cg.	Subcutaneous.	killed 70 days. Generalized tuberculosis.
Guinea-pig 44	350 gm.	June 7, '07	½ c.c. stand. susp.	Intraperitoneal.	d. 21 days. Generalized tuberculosis.
Guinea-pig 147	470 gm.	April 3, '08	½ c.c. " "	Intraperitoneal.	d. 11 days(?) of same.
Guinea-pig 210	445 gm.	Aug. 13, '08	½ c.c. " "	Intraperitoneal.	d. 17 days. Generalized tuberculosis.

Bacteriological Diagnosis.—B. tuberculosis, bovine type.

Case 07.9.—The first culture on egg medium grew well but not luxuriantly. The first transfer to glycerine agar grew, although feebly. The third generation on glycerine agar was approximately as vigorous as a fresh culture of human type. The transfer to glycerine bouillon was somewhat more readily accomplished than in Case 07.8, and the character of growth was like that described for 07.8.

Reaction on Glycerine Bouillon.—3 different lots of bouillon were used as follows:

I. Sept. 23, '07, inoc. Glyc. Bou. 273, acidity 2.1 % (25 c.c. bouillon in 100 c.c. Ehrlenmeyer flask.) Oct. 13, '07, surface covered with a delicate, smooth, translucent membrane with a thick island at the point of inoculation. Acidity 0.35 %. Nov. 13, '07, membrane thicker, even with fine wrinkles, white, glistening. Acidity 2.0 %. Jan. 2, '07, membrane same. Acidity 2.6 %.

II. Oct. 13, '07, inoc. from Flask I to Glyc. Bou. 211. Acidity 1.1 %. 100 c.c.

bouillon in 250 c.c. Ehrlenmeyer flask. Nov. 1, '07, surface almost covered with a smooth, thin, translucent membrane. A somewhat thicker island at point of inoculation. Reaction neutral. Nov. 29, '07, membrane moderately heavy, evenly wrinkled, white and glistening. Acidity 1.1 %. Jan. 2, '07, membrane same. Acidity 2.4 %.

III. Dec. 2, '08, inoc. Glyc. Bou. R. I. 4. Acidity 1.5 %. Layer 1 in. deep in 100 c.c. flask with 20th generation on glycerine agar. Jan. 21, '09, thin translucent membrane with thicker opaque white veins and one thick warty island covers surface and has begun to extend up sides of flask. Acidity 0.8 %. Feb. 8, '09, membrane somewhat thicker. Acidity 0.4 %. March 2, '09, membrane same. Acidity 0.7 %.

At the end of the first two tests, the cultures looked pure, but no microscopic examination was made. After the third test the culture was proven pure.

The virulence test with this culture resulted as follows:

Animal.	Weight.	Date.	Dose.	Injection site.	Result.
Rabbit 266	2110 gm.	June 7, '07	½ c.c. stand. susp.	$\frac{1}{10}$ Intravenous.	d. 17 days. Generalized tuberculosis.
Rabbit 276	2150 "	Apr. 3, '08	½ c.c. " "	"	d. 23 days. Generalized tuberculosis.
Rabbit 341	1800 "	June 16, '08	$\frac{1}{100}$ c.c. " "	"	d. 42 days. Generalized tuberculosis.
Guinea-pig 148	670 "	Apr. 3, '08	½ c.c. " "	Intraperitoneal.	d. 11 days. Generalized tuberculosis.
Guinea-pig 43	350 "	June 7, '07	½ c.c. " "	"	d. 25 days. Generalized tuberculosis.

Bacteriological diagnosis.—B. tuberculosis, bovine type.

Case 07.11.—Cultures were obtained on egg medium. This culture grew fairly well in the first and subsequent generations. In spite of repeated trials, no successful transfers to glycerine agar or glycerine bouillon have been ob-

Animal.	Weight.	Date.	Injection site.	Dose.	Result.
Rabbit 306	1640 gm.	Oct. 4, '07	Intravenous.	½ c.c. stand. susp.	d. 21 days. Generalized tuberculosis.
Rabbit 311	2650 "	Feb. 14, '08	Intravenous.	± $\frac{1}{10}$ c.c. " "	d. 26 days. Generalized tuberculosis.
Rabbit 336	3330 "	May 18, '08	Intravenous.	± $\frac{1}{200}$ c.c. " "	d. 27 days. Generalized tuberculosis.
Rabbit 339	2315 "	June 18, '08	Subcutaneous.	5 c.c. " "	Killed 7 weeks. Generalized active tuberculosis.
Guinea-pig 79	520 "	Oct. 4, '07	Intraperitoneal.	½ c.c. " "	d. 1 mo. Generalized tuberculosis.
Guinea-pig 124	580 "	Feb. 14, '07	Intraperitoneal.	± $\frac{1}{10}$ c.c. " "	d. 3 weeks. Generalized tuberculosis.

tained. From the third generation on egg medium, a successful transfer was made to dog serum. The third dog serum generation was less strong than the second, perhaps due to a fault in the serum. This medium could not thereafter be obtained. The culture was continued on egg medium, growing moderately well until Aug. and Sept., '08. Then a transfer almost failed to grow and since then growth has been very feeble. The virulence tests were made from egg medium or dog serum during the period of most vigorous growth. The results are shown by the foregoing table.

In those cases where the dosage is indicated as ±, the suspension was too thin for the standard and the dose given is an estimate.

Bacteriological diagnosis: B. tuberculosis, bovine type.

Case 07.53.—Cultures on egg medium grew readily from the first. Transfer from the first generation on egg medium to glycerine agar was successful although the growth was not vigorous. The third glycerine agar generation grew as vigorously as the first generation of a culture of the human type. Put on glycerine bouillon in Sept., 07, it grew slowly at first into a small thick white island at the inoculation site. There gradually spread out a thin, veil-like membrane with thicker veins. This thickened slightly in the following weeks. Later growths on glycerine bouillon were of the same general character except that the membrane thickened in the second year of cultivation.

Titration Tests.—I. Sept. 23, '07, inoc. Glyc. Bou. 273 (reaction 2.1 % acid). 2 flasks. Thin layers in small flasks. Nov. 29, '07, a thin membrane with one thick spot quite covers the surface of each flask. Reaction (*a*) 1.9 % acid, (*b*) 1.7 % acid.. Flask (*a*) developed a growth of mould after being opened. Flask (*b*) Jan. 1, '08, membrane somewhat thicker. Reaction, 2.0 % acid. Feb. 12, '08, membrane is slightly thicker. Reaction 3.1 %.

Animal.	Weight.	Date.	Dose.	Injection site.	Result.
Rabbit 299	2070 gm.	Oct. 26, '07	½ c.c. stand. susp.	Intravenous.	d. 20 days. Generalized tuberculosis.
Rabbit 291	2450 gm.	April 3, '08	½ c.c. " "	Intravenous.	d. 24 days. Generalized tuberculosis.
Rabbit 337	2190 gm.	June 18, '08	$\frac{1}{200}$ c.c. " "	Intravenous.	d. 35 days. Generalized tuberculosis.
Calf 206	230 lb.	Aug. 12, '08	5 mg.	Intravenous.	d. 17 days. Generalized tuberculosis.
Calf 205	190 lb.	Aug. 12, '08	5 cg.	Subcutaneous.	Killed 70 days. Generalized tuberculosis.
Guinea-pig 86	400 gm.	Oct. 26, '07	½ c.c. " "	Intraperitoneal.	d. 30 days. Generalized tuberculosis.
Guinea-pig 149	360 gm.	April 3, '08	½ c.c. " "	Intraperitoneal.	d. 20 days. Generalized tuberculosis.
Guinea-pig 209	425 gm.	Aug. 13, '08	½ mg.	Intraperitoneal.	d. 26 days. Generalized tuberculosis.

II. Feb. 1, '08, inoc. Glyc. Bou. 304 (reaction 2.0 % acid) from flask (*b*) of previous test. March 10, '08, the inoculation point has thickened to a yellowish-white island 1 cm. in diam. From this during the past week a thin translucent membrane has rapidly extended over the surface which is now just covered. Reaction 1.9 % acid. June 3, '08, the membrane is somewhat thicker, rather irregular and dry. Reaction 1.6 % acid. Discarded.

III. Nov. 30, '08, inoc. Glyc. Bou. (4) acidity 1.5 % 1 in. layer in 200 flask. Jan. 3, '09, surface just covered with generally thin translucent membrane. Reaction = .6 % acid. Jan. 21, '09, membrane somewhat thicker. Reaction = .4 % acid. Feb. 8, '09, membrane same. Reaction = .5 % acid.

Virulence tests with this culture gave the foregoing results (see table).

Bacteriological diagnosis.—B. tuberculosis, bovine type.

Case 07.54.—Cultures were readily obtained on egg medium. Transfers from the first generation on egg medium to glycerine agar were entirely successful, the culture growing vigorously in the first and subsequent generations. Readily transferred to glycerine bouillon but the culture has never grown rapidly. Starting from the inoculation flask the culture extends very slowly, thickening almost as rapidly as it grows over the surface to a thick irregular curdy membrane. The culture tends to extend by putting out veil-like wings, 5 mm. in width, which thicken before others start. Inoculated on a rather thin layer and a large surface of bouillon, the culture rarely covers the surface after several months.

Titration Tests.—I. Sept. 23, '07, inoc. 3 week glycerine agar generation to Glyc. Bou. 273 (reaction 2.1 % acid). Nov. 29, '07, irregular curdy membrane partly covers surface. Reaction .75 % acid. Jan. 2, '08, irregular thick membrane entirely covers surface. Reaction .4 % alk. Feb. 12, '08, membrane of same character but slightly thicker. Reaction 2.0 % acid.

II. Nov. 29, '07, inoc. Glyc. Bou. 304 (reaction 2.0 %) from Flask 1. Jan. 2, '08, moderately thick rough membrane nearly covers surface. Reaction .6 % acid. Feb. 1, '08, heavy white glistening membrane fully covers surface. Reaction neutral. Discarded.

III. Feb. 1, '08, inoc. 5th glycerine agar culture on Glyc. Bou. 304. Reaction 2.0 % acid. April 2, '08, heavy opaque wrinkled membrane with very thin growing margin, some half cover the surface. Reaction 0.7 % acid. June 3, '08, surface covered with a heavy warty membrane having a few niches filled with very thin membrane. Reaction .6 % acid.

IV. June 5, '08, inoc. the 13th glycerine generation to Glyc. Bou. 347, 2 flasks. Reaction 1.9 % acid. Flask (*a*). July 15, '08, surface one-third covered with white opaque pearly membrane. Reaction .4 % acid. Aug. 11, '08, membrane somewhat larger. Reaction .3 % acid. Flask (*b*). Aug. 9, '08, surface ¾ covered with a thick curdy membrane with some thin marginal areas. Reaction 1.8 % acid. Sept. 10, '08, membrane almost covers surface. Reaction .2 % alkaline.

The culture was tested for virulence from glycerine agar with the following results:

Rabbit 295.—1830 gm. Oct. 26, '07, ½ c.c. stand. susp. Intravenous. Killed Feb. 6, '08. Localized tuberculosis in iris, lungs, kidneys, and right testicle.

Guinea-pig 87.—500 gm. Oct. 26, '07, ½ c.c. stand. susp. Intraperitoneal. Died 24 days. Generalized tuberculosis.

Bacteriological diagnosis.—B. tuberculosis, human type.

CASE 07.55.—Cultures on egg medium grew readily. Transfer from the first generation to glycerine agar grew luxuriantly. Good but slow growth on glycerine bouillon. The membrane thickened as it spread, becoming wrinkled, warty and opaque.

Titration Tests.—I. Nov. 11, '07, inoc. Glyc. Bou. 273 (reaction 2.1 % acid) from the fourth glycerine agar generation. There was but slight growth until March 1, '08, when it began and progressed steadily. April 2, '08, moderately thick semi-translucent membrane with irregular margins almost covers the surface. Reaction 5.4 % acid.

II. June 5, '08, the eleventh glycerine agar generation was transferred to Glyc. Bou. 347. Reaction 1.9 % acid. Aug. 11, '08, surface ⅝ covered with a very heavy wrinkled membrane. Reaction 3.4 % acid.

Tested for virulence, the culture gave the following results:

Animal.	Weight.	Date.	Dose.	Injection site.	Result.
Rabbit 307	1700	Oct. 4, '07	½ c.c. stand. susp.	Intravenous.	Killed 3 months. In apparently good health at time. Autopsy: localized chronic tuberculosis in iris, lungs, and kidneys.
Guinea-pig 78	460	Oct. 4, '07	½ c.c. " "	Intrape r toneal.	Died 27 days. General miliary tuberculosis.

Bacteriological diagnosis.—B. tuberculosis, human type.

CASE 07.56.—Cultures on egg medium grew well in the first generation. Transferred at this time to glycerine agar, the growth was luxuriant and continued so in all subsequent generations. Grew well although not rapidly on glycerine bouillon. The membrane thickened as it grew, resulting in a wrinkled, opaque, rather yellowish surface with thin translucent narrow growing margin.

Titration Tests.—I. Nov. 11, '07, inoc. Glyc. Bou. 273. Reaction 2.1 % acid. Feb. 12, '08, a heavy wrinkled membrane half covers the surface. Reaction 1.9 % acid. April 2, '08, heavy wrinkled membrane covers the surface and extends up the sides of the flask. Reaction 3.8 % acid.

II. Feb. 1, '08, transfer from Flask No. 1 to Glyc. Bou. 304. Reaction 2.0 %. April 2, '08, heavy wrinkled membrane with a narrow thin margin ¾ covers surface. Reaction 1.9 % acid. June 3, '08, surface is ⅝ covered with a thick evenly wrinkled moist membrane. Reaction 4.3 %.

Virulence tests with this culture gave the following results:

Rabbit 308, wt. 1730 gm. Received ½ c.c. stand. susp. intravenously on Oct. 4, '07. Killed when in apparently good health after 4½ months. Chronic localized tuberculosis was found in the ear at inoculation site, mammary glands in thoracic region, and both kidneys.

Guinea-pig 77, wt. 470 gm. Received Oct. 4, '07, ½ c.c. stand. susp. intraperitoneally. Died 1 month. Generalized tuberculosis.

Bacteriological diagnosis.—B. tuberculosis, human type.

CASE 07.61.—Cultures were readily obtained on egg medium. They grew well and from the first generation on this medium transfers were made to glycerine agar. These grew luxuriantly, as did all cultures on this medium, for a number of months. In Feb., '08, through accident the culture failed to grow but was recovered by passing older cultures through another guinea-pig. The titration test recorded was done subsequent to this passage through the second animal. The virulence test was done during the first period when the culture was growing vigorously. Transferred to glycerine bouillon, the culture grew slowly but steadily, thickening as it spread. The character of membrane was as in Cases 07.54, 07.55, 07.56, and need not be described in detail.

Titration Test.—Jan. 7, '09, inoc. Glyc. Bou. 4. Reaction 1.5 % acid. Feb. 19, '09, for 2 or 3 days surface has been covered with a moderately thick, uneven, opaque, white membrane. Reaction .5 % acid. March 20, '09, membrane more evenly thickened. Reaction 1.8 % acid.

Virulence test with the culture gave the following result:

Rabbit 309, wt. 1620 gm. Oct. 4, '07, received ½ c.c. stand. susp. intravenously. 3 months later animal was in good condition (wt. 2370). Killed. Had chronic localized tuberculosis in the kidneys.

Guinea-pig 76, wt. 395 gm. Oct. 4, '07, received ½ c.c. stand. susp. intraperitoneally. Died 1 month. Generalized tuberculosis.

Bacteriological diagnosis.—B. tuberculosis, human type.

CASE 07.73.—Cultures on egg medium grew well but slowly. The first generation was successfully transferred to glycerine agar growing with moderate vigor. The third glycerine agar generation grew with about the same vigor as a fresh culture of human bacilli. The seventh glycerine agar generation grew readily on glycerine bouillon. The inoculation spot thickened and spread slowly for some weeks, when rather quickly there spread out from it a thin translucent membrane extending quickly to and up the sides of the flask. This peripheral membrane thickened slightly in vein-like lines. Subsequent growths did likewise.

Titration Tests.—I. Feb. 24, '08, inoc. Glyc. Bou. 304. Reaction 2.0 % acid, with 7th glycerine agar generation. April 29, '08, surface just covered. Reaction .9 % acid. June 3, '08, membrane somewhat thicker. Reaction 2.1 % acid. Culture macroscopically pure.

II. June 5, '08, inoc. Glyc. Bou. 347. Reaction 1.9 % acid. July 15, '08, surface has been covered for 2 weeks. Reaction .5 % acid. Aug. 11, '08, reaction .9 % acid.

III. July 10, '08, inoc. Glyc. Bou. 364. Reaction 2.0 % acid, with the 13th glycerine-agar generation. July 30, '08, surface just covered. Reaction .8 % acid.

IV. July 10. '08, inoc. Glyc. Bou. 364. Reaction 2.0 % with the thirteenth glycerine-agar generation. July 30, '08, surface covered. Aug. 9, '08, membrane thicker. Reaction .4 % acid. Aug. 28, '08, reaction .3 % alk. Sept. 21, '08, reaction .3 % alk.

V. Dec. 2, '08, inoc. Glyc. Bou. 4. Reaction 1.5 % acid. Feb. 8, '09, surface has been covered for four or five days. Reaction 1.0 % acid. March 2, '09, membrane thicker. Reaction .6 %.

The virulence of the culture was determined with the following result:

Animal.	Weight.	Date.	Dose.	Injection site.	Result.
Rabbit 276	1680 gm.	Oct. 26, '07	½ stand. susp.	Intravenous.	Died 16 days. Generalized tuberculosis.
Rabbit 334	1850 "	May 15, '08	$\frac{1}{200}$ " "	"	Died 7 weeks. Generalized tuberculosis.
Guinea-pig 88	380 "	Oct. 26, '08	½ " "	Intraperitoneal.	Died 15 days. Generalized tuberculosis.
Guinea-pig 208	470 "	Aug. 13, '08	½ " "	"	Died 17 days. Generalized tuberculosis.
Calf 204	210 lb.	Aug. 12, '08	5 mg.	Intravenous.	Died 20 days. Generalized tuberculosis.
Calf 202	200 "	Aug. 12, '08	5 cg.	Subcutaneous.	Killed 70 days. Generalized tuberculosis.

Bacteriological diagnosis.—B. tuberculosis, bovine type.

Case 07.74.—Cultures were readily obtained on egg medium. The first generation from this medium grew luxuriantly on glycerine agar, as did all subsequent subcultures on the agar. On glycerine bouillon, the growth was slow but constant. The growth on this medium corrsponded to that described for Culture 07.55.

Titration Tests.—I. Nov. 11, '07, inoc. Glyc. Bou. 273. Reaction 2.1 % acid. April 2, '08, very irregular membrane covers $\frac{1}{10}$ of surface. Reaction 2.9 % acid.

II. April 2, '08, inoc. from Flask 1. to Glyc. Bou. 328. Reaction 1.9 % acid. June 3, '08, membrane just covers surface. Reaction 3.9 % acid.

III. June 5, '08, inoc. Glyc. Bou. 347. Reaction 1.9 % acid with the eleventh glycerine agar generation. July 15, '08, surface ⅞ covered. Reaction .7 % acid. Aug. 15, '08, membrane completely covers surface. Reaction 3.4 % acid.

The virulence of this culture for rabbits was tested as follows:

Rabbit 343.—Wt. 1880 gm. June 16, '08, received ½ c.c. stand. susp. intravenously. 2 months later had gained much weight and was in good condition. Killed. Localized chronic regressive tubercles were found in the lungs. There was localized tuberculosis as well in kidneys, liver, and spleen.

Bacteriological diagnosis.—B. tuberculosis, human type.

Case 07.79.—Cultures were readily obtained on egg medium and subsequent subcultures on this medium grew very well. The transfers of the first and second generations on egg medium to glycerine agar failed entirely. Subcultures from the third generation gave a feeble growth. Subsequent subcul-

tures from glycerine agar to glycerine agar were always successful but the growth was at first feeble. The sixth glycerine agar generation was about as vigorous as that of a first subculture of the human bacillus. Transfer from the third glycerine agar generation to glycerine bouillon grew very little for a month and after that spread quickly over the surface. The resulting membrane was similar to that already described for Case 07.9 and has been so at all subsequent trials.

Titration Tests.—I. April 25, '08, inoc. third glycerine agar culture on Glyc. Bou. 328. Reaction 1.9 % acid. June 3, '08, thin, even, smooth or finely granular, translucent membrane with one small thick wrinkled spot just covers surface. Reaction .5 % acid. July 2, '08, membrane somewhat thicker. Reaction .8 % acid.

II. July 10, '08, inoc. sixth glycerine agar generation on Glyc. Bou. 364. Reaction 2.0 %. July 27, '08, surface just covered by membrane. Reaction 1.4 % acid. Aug. 9, '08, membrane thicker. Reaction 1.1 % acid. Nov. 10, '08, reaction 0.1 % acid.

Tested for virulence this culture gave the following results:

Animal.	Weight.	Date.	Dose.	Injection site.	Result.
Rabbit 290	2010	Oct. 26, '07	½ c.c. stand. susp.	Intravenous (lost nearly all)	Died 7 weeks. Generalized tuberculosis.
" 333	1800	May 15, '08	$\frac{1}{200}$ c.c. " "	Intravenous.	Died 7 weeks. Generalized tuberculosis.
Guinea-pig 89	390	Oct. 26, '07	½ c.c. " "	Intraperitoneal.	Died 7 weeks. Generalized tuberculosis.

Bacteriological diagnosis.—B. tuberculosis, bovine type.

CASE 07.86[2].—Cultures on egg medium grew readily. The first generation culture on this medium was successfully transplanted to glycerine agar. The first glycerine agar generation grew as vigorously as subsequent ones. Growth was readily obtained on glycerine bouillon. The membrane formed was thick yellowish-white, spreading slowly and thickening as it spread.

Titration Test.—March 17, '08, inoc. the eighth glycerine agar generation on Glyc. Bou. 328. Reaction 1.9 % acid. June 3, '08, thick, wrinkled, dry membrane covers the surface and sides of the flask. Shows some tendency to fall. Reaction 4.5 % acid.

Virulence test with this culture gave the following results:

Rabbit No. 298, wt. 2200 gm. Dec. 10, '07, received ½ c.c. stand. susp. intravenously. After 3 months animal had gained 450 gm. in wt. Good condition. Killed. Chronic localized tuberculosis was found in the lungs and kidneys.

Guinea-pig No. 113, wt. 370 gm. Dec. 10, '07, received ½ c.c. stand. susp. intraperitoneally. Died 24 days. Generalized tuberculosis.

Bacteriological diagnosis.—B. tuberculosis, human type.

CASE 07.92.—Cultures on egg medium grew well from the beginning. An

[2] While the disease in this case involved the axillary glands it was so apparently successive to primary disease of the cervical glands that it seems proper to include the case in this series.

attempt to transfer the first egg-medium generation to glycerine agar failed. The second egg medium generation transferred to glycerine agar gave a feeble growth which was, however. successfully maintained in subcultures on this medium. The sixth glycerine agar generation was the first in which the vigor of growth was approximately that of a fresh culture of human type. The fourth generation on glycerine agar inoculated on glycerine bouillon grew slowly for 3 months to a thick yellow-white island about 1 cm. across. Then it began to spread rapidly and in 2 weeks the surface was covered with a thin translucent membrane. This later extended up the sides of the flask and thickened slightly. In subsequent transfers, the character of growth on glycerine bouillon was the same.

Titration Test.—I. April 9, '08, inoc. Glyc. Bou. 328. Reaction 1.9 % acid. June 3, '08, very slight growth at inoculation point. July 15, '08, surface ¾ covered with a very thin, translucent membrane in which are 2 thicker islands. Reaction 1.7 % acid. Aug. 9, '08, surface covered. Membrane somewhat thicker. Reaction .6 % acid. Sept. 10, '08, membrane about same. Reaction .2 % acid.

II. Nov. 30, '08, inoc. Glyc. Bou. 4 (reaction 1.5 % acid). Feb. 8, '09, surface almost covered. Membrane character as before. Reaction .3 % acid. March 2, '09, surface almost covered. Reaction .6 % acid.

Virulence tests with this culture gave the following results:

Animal.	Weight.	Date.	Injection site.	Dose.	Result.
Rabbit 297	1860 gm.	Oct. 26, '07	Intravenous.	½ c.c. stand. susp.	Died 18 days. Generalized tuberculosis.
Rabbit 294	2190 "	May 18, '08	"	$\frac{1}{200}$ c.c. " "	Died 35 days. Generalized tuberculosis.
Guinea-pig 90	600 "	Oct. 26, '07	Intraperitoneal.	½ c.c. " "	Died 10 days (?) of spontaneous disease. Tubercle just visible in spleen and lungs.

Bacteriological diagnosis.—B. tuberculosis, bovine type.

CASE 07.93.—Cultures were readily obtained on egg medium. The first subcultures grew, although never vigorously. Repeated attempts to get growth on glycerine agar and glycerine bouillon by transfer from the egg medium cultures failed entirely. The culture was maintained on egg medium for 12 generations, when it was lost through accident. In the meantime, its virulence had been tested with the results shown in the following table (page 95).

Bacteriological diagnosis.—B. tuberculosis, bovine type.

CASE 07.94.—Cultures on egg medium were readily obtained. They grew well from the beginning. Subcultures from the first generation to glycerine agar gave a very fair growth which was maintained. The fifth glycerine agar generation gave as vigorous a growth as a fresh culture of human type. Transfer from the fifth and subsequent glycerine agar generations to glycerine bouillon gave good growths. The membrane either spreads at once as a thin

Animal.	Weight.	Dose.	Injection site.	Date.	Result.
Rabbit 274	2150 gm.	½ c.c. stand. susp.	Intravenous.	Dec. 10, '07	Died 17 days. Generalized tuberculosis.
Rabbit 332	2340 "	$\frac{1}{200}$ c.c. " "	"	May 18, '08	Killed at 2 months. Has lost much weight in the last 2 weeks and was suffering from an actively progressing, generalized tuberculosis.
Guinea-pig 112	350 "	½ c.c. " "	Intraperitoneal.	Dec. 10, '07	Died 18 days. Generalized tuberculosis.

translucent film over the whole surface or the inoculation point thickens to a yellowish-white island .5 to 1 cm. across, and from this a thin membrane suddenly spreads over the whole surface. When the cultures became older, the thinner parts of the membrane became thicker in small spots and vein-like lines.

Titration Test.—I. March 17, '08, inoc. Glyc. Bou. 328. Reaction 1.9 % acid, with the fifth glycerine agar generation (2 flasks). April 8, '08, surface of flasks is just covered. Flask (*a*) has reaction .9 % acid. June 3, '08, flask (*b*) membrane has largely fallen and been replaced by a second thinner film. Reaction 1.2 % acid.

II. June 5, '08, inoc. Glyc. Bou. 347. Reaction 1.9 % acid with the eighth glycerine agar generation. July 15, '08, surface ¾ covered. Reaction .7 % acid. Aug. 11, '08, surface covered. Reaction .7 % acid.

III. July 10, '08, inoc. Glyc. Bou. 364. Reaction 2.0 % acid, with the ninth glycerine agar generation. July 30, '08, surface just covered. Reaction 1.1 % acid. Aug. 9, '08, membrane thicker. Reaction .3 % acid. Sept. 10, '08, reaction .1 % acid.

IV. Dec. 2, '08, inoc. Glyc. Bou. 4. Reaction 1.5 % acid. 3 inch layer in a 500 c.c. Ehrlenmeyer flask with the twelfth glycerine agar generation. Jan. 21, '09, surface ⅔ covered. Reaction .1 % acid. Feb. 8, '09, surface covered. Reaction .4 % acid. March 2, '09, membrane thicker. Reaction .9 % acid.

V. Dec. 2, '08, inoc. same culture as in (4) in same bouillon but 1½ inch

Animal.	Weight.	Date.	Dose.	Injection site.	Result.
Rabbit 264	3270	Dec. 10,'07	½ c.c. stand. susp.	Intravenous.	Died 17 days. Generalized tuberculosis.
Rabbit 331	1450	May 18,'08	$\frac{1}{200}$ c.c. " "	"	Died 5 weeks. Generalized tuberculosis.
Guinea-pig 114	400	Dec. 10,'07	½ c.c. " "	"	Died 2 months. Generalized tuberculosis.

layer in 200 c.c. flask. Jan. 21, '09, surface covered. Reaction .6 % acid. Feb. 8, '09, membrane thicker. Reaction .6 % acid. March 2, '09, membrane same. Reaction .7 % acid.

Virulence tests with this culture gave the results shown in the foregoing table.

Bacteriological diagnosis.—B. tuberculosis, bovine type.

DIFFERENTIAL CHARACTERISTICS.

Before proceeding to collect and analyze the results as set forth in the foregoing protocols, it will be well to review briefly the characteristics which it is necessary to consider in the differentiation of mammalian tubercle bacilli into the two types, human and bovine. Theobald Smith (7) in 1898 first pointed out that the bacilli derived from tuberculous tissue of cattle differed in fundamental particulars from those derived from cases of human pulmonary tuberculosis. The observations recorded at that time have been repeatedly made since by workers in all countries. Somewhat extended and modified by later experience, the differentiation today is reached by developing the following points:

Grown under special conditions, that is on coagulated dog serum, the recent isolations differ in morphology. The bovine bacilli are short, straight, plump, and stain solidly. The human bacilli are longer, frequently curved, thinner, and when stained are often beaded. The initial cultivation of the tubercle bacillus is more difficult from the bovine than from the human source. Grown on glycerine bouillon, there are constant differences in the character of the growth. The bacilli from the human disease grow as a thick wrinkled membrane from the beginning which tends to spread rather slowly, and thickens about as quickly as it spreads. A favorable culture of bovine origin will spread rapidly as a thin translucent membrane over the whole surface. If the culture has been long under cultivation, the membrane may thicken evenly till it is not to be distinguished from the completed human membrane. If recently isolated, either this thickening does not occur or it develops in irregular lines or spots.

Finally, the bacilli differ widely in virulence. The inoculation disease from the bovine bacillus in young cattle tends always to become general. Inoculated intravenously in small doses, the cattle usually die of acute miliary tuberculosis. If human bacilli are inoculated in similar dose intravenously, local chronic disease results at most. If the human bacilli are inoculated intraperitoneally, intrapleurally, or subcutaneously, even in large dose, only a local chronic disease results. The virulence of the two bacilli for rabbits was found to be exactly the same as for cattle.

In 1903, Theobald Smith (8) published the further observation, that corresponding to the qualitative differences between the character of the growth of the two types of bacilli on glycerine bouillon,

there are characteristic differences in the amount of acid and alkali production on this medium. It has proven to be a matter of some difficulty in other hands to develop consistently these changes in chemical reaction, and in such fashion as to aid in the classification of cultures. I have studied the reaction changes produced by all the cultures in this series, but factors unforeseen and probably extraneous, it seems to me, have led to marked irregularity in the results. For this reason I shall, in the following pages, consider the grouping of the cultures according to the characters first given above, virulence for rabbits and cattle, growth character on glycerine bouillon, and adaptability in artificial cultivation. Subsequently, in a separate section, I shall discuss the reaction changes on glycerine bouillon.

Virulence.—In dealing with cultures of the tubercle bacillus recently isolated, the character which must be deemed of first importance in an attempt to classify by type is that of virulence. The animal of choice would be the calf. As stated above, however, it has been abundantly demonstrated that the virulence for rabbits is a perfect index of that for calves. Consequently, rabbits have been selected for most of the tests.

Each culture, as soon as possible after a vigorous growth had been established, was inoculated intravenously into a rabbit in doses of one-half cubic centimeter of the standard suspension (approximately .5 milligram). Nine of the cultures killed the rabbits in from two to three weeks with lesions of generalized tuberculosis. Six cultures did not kill. The animals inoculated with the latter cultures ultimately gained in weight, were chloroformed after two to four months, and showed at autopsy a small or moderate grade of localized chronic tuberculosis. Several of the nine cultures which killed were retested in the same dose to exclude the possibility of accidental, coincidental disease. The result was as before. Finally each of the nine virulent cultures was inoculated intravenously in 1/100th of the standard dose, corresponding to about 1/200 milligram of culture. This dose killed in several instances in less than twenty-five days; eight of the animals were dead by the fortieth day. The ninth rabbit survived two months, failed rapidly, was killed and found to have an active, generalized, progressive tuberculosis.

Nine of the fifteen cultures studied were thus found to be viru-

lent for rabbits. Six cultures were not virulent for this animal. It will be noticed that the difference in virulence was a positive one. For the non-virulent group, one-half cubic centimeter of the standard suspension was a dose which failed to kill, and judging from the reaction and the lesions in the surviving animals it was considerably below the fatal dose. One one-hundredth of this amount was found to be certainly fatal in the case of the virulent group. The animal which lived two months after inoculation was killed because of extraneous circumstances. It was failing rapidly and the disease at autopsy was found to be general and progressing, and the animal could not have survived much longer (see Case 07.92). The grouping of the cultures in respect to virulence is shown in the following table. The numbers refer to the case number in the corresponding protocol.

Virulent for rabbits.	Non-virulent for rabbits.
07.8	07.54
07.9	07.55
07.11	07.56
07.53	07.61
07.73	07.74
07.79	07.86
07.92	
07.93	
07.94	

It has been abundantly shown that a corresponding virulence for cattle can be assumed. However, three of these cultures (07.8, 07.53, 07.73) which attracted our attention by reason of apparent abnormalities in reaction on glycerine bouillon were tested on calves. Three calves were inoculated intravenously with five milligrams of each of these cultures. All died in less than three weeks and at autopsy showed an acute, generalized, miliary tuberculosis.

One calf was inoculated in each case with five centigrams of culture subcutaneously in the region of the neck. They were killed seventy days later. There was found at autopsy in each case a large tuberculous lesion at the site of inoculation. The lymphatic gland anterior to the shoulder was much enlarged and tuberculous. Other glands in the same chain were more or less involved. Small tubercles were found in moderate numbers in the internal organs of all the

HENRY F.
VAUGHAN
LIBRARY

animals. These three cultures, then, were proved virulent for calves; and the experiments afford additional support to the existing evidence that virulence of mammalian tubercle bacilli, for rabbits and cattle go hand in hand.

Cultural Characters.—The six cultures which failed to kill rabbits were, in each instance, transferred at the first subculture to glycerine agar and from the beginning grew luxuriantly on this medium. They were as easily transferred to glycerine bouillon, growing for the most part with a heavy membrane which spread slowly and thickened markedly as it spread. The nine cultures virulent for rabbits have shown a very variable adaptability to culture medium. Subcultured each month to six weeks, I shall give the number of the subculture on glycerine agar at which they have attained practically the luxuriance of growth of the non-virulent cultures as an index of their adaptability. Four cultures grew at once on glycerine agar and at the third subculture were as vigorous as the others. Three cultures failed to grow on the first glycerine agar transfer and required from five to six subcultures to reach maximum vigor. Two cultures, in spite of repeated trials over a period of eighteen months, still refused to grow on glycerine agar or glycerine bouillon. These cultures then failed to grow on two successive lots of egg medium. One of them was entirely lost, the other probably cannot be recovered. The seven cultures which were successfullly transferred to glycerine agar also grew well from about the same period on glycerine bouillon.

When they started to grow on this medium soon after the inoculation of the flask, they spread at once as a thin translucent membrane over the whole surface. In the earlier generations, this membrane thickened only irregularly. Some of the later generations have given completed membranes only slightly less thick than those of the non-virulent cultures. When the culture in any flask started more slowly there was always formed a thick wrinkled island one-half to one centimeter in diameter from which there would rather suddenly extend a thin translucent film to cover the surface.

For the reason that dog serum was not regularly obtainable during the earlier period of this study, I have devoted only small attention to the morphological characters of the two groups of bacilli,

and have not recorded in detail the results of such examinations as I made.

In their adaptability to artificial cultivation, and in the character of the growth on glycerine bouillon, the cultures have consistently shown differences entirely in accord with the differences in virulence. The non-virulent strains were readily established in culture, growing with a thick, slowly extending membrane on glycerine bouillon. The virulent cultures were established in culture with more or less difficulty, and in two instances with imperfect success. It seems, therefore, safe to conclude that the nine cultures of lesser adaptability and high virulence are of bovine type, and the six cultures which readily adapt themselves to glycerine agar and which have low virulence for rabbits are of human type.

It should be noted that two of the cultures (07.11–07.93) were more difficult to cultivate than the usual culture of bovine origin being definitely atypical in this respect.

Reaction Curves on Glycerine Bouillon.—In previous paragraphs, I have stated that one of the constant differences between the human and bovine type of mammalian tubercle bacilli, according to Theobald Smith, lies in the change in the chemical reaction of glycerine bouillon, when the bacilli are grown on this medium. Starting with an initial acidity of about 2 per cent., phenolphthalein being the indicator, the first change in either instance is to diminish the amount of acid. In the case of the bovine type, the reaction may even become alkaline. In the case of the human type, the neutral point is more or less closely approached but is seldom passed. The time when the acidity is least usually corresponds to the period of growth when the surface has just been completely covered in the case of a culture growing rapidly. From this point, the human type of culture rapidly returns toward the initial acidity and often goes beyond it. In the case of the bovine type, there may be, and usually is, some increase in acidity but this is most often slight and is sometimes lacking. In a later publication by Smith and Brown, this distinction was maintained, and it was pointed out that there were constant differences between cultures of the human type, some returning to an acidity of about 2 per cent., others tending to return to about 4 per cent. of acid as an end reaction.

In the description of this procedure certain difficulties were pointed out and certain limitations put upon its use as an absolute test for type. The cultures must be maintained free from fungi and other bacteria during the whole period of observation. Certain preparations of bouillon are unfavorable and, consequently, controls of well-known cultures must be carried in every series in which a new lot of medium is used. The cultures must have become perfectly adapted to the medium in order to give regularly progressing growth and typical curves of reaction.

In the hands of other observers, this method as a differential test has given rise to various opinions.

Beitzka (9) thinks it is of no value. One feels, however, that he worked without due regard to the limitations originally laid down.

The English Commission workers (10) thought that the figures of Smith showed no definite line of demarcation, nor did their own. Their results seemed to show that the bovine bacillus when growing to produce as heavy a membrane as the human type would give an acid reaction. Smith's figures, taken as a whole, do not lend themselves to the interpretation they put on them. Moreover, the most characteristic reaction of the bovine group were obtained by Smith with cultures Bov. I and III which had been very long under cultivation and which grew with the vigor of human cultures.

Bang (11) found that the lines were maintained in the case of certain stock cultures of either type and that the avian tubercle bacillus gave a reaction curve like that of the bovine type.

Duval (5) used the test in the study of four cultures from human source without presenting fresh evidence as to its validity. His work emphasizes the difficulty of obtaining consistent results with a few tests on freshly isolated cultures. He found one culture (IV) which while having all the other characteristics of a culture of human type, gave an initial fall in reaction to the neutral point or near it with no secondary rise in acidity. He presents some evidence to show that the secondary rise in acidity is due to an autolysis of the human type of bacilli in the presence of glycerine, irrespective of growth.

Work in other hands has obviously done little to establish the

test on a firm footing, and has, on the other hand, raised no serious criticism of it.

In classifying the cultures of my series in one or the other type, I have left to one side the evidence of the reaction curves for several reasons. In the first place, in the case of many of the earlier tests, I am not certain of the purity of the cultures throughout the period of examination. I relied on the macroscopic appearance of the cultures as an evidence of continued purity during the first year of the work. At this time several of the cultures of high virulence gave frankly acid end reactions. Later some flasks were left to be finished as I was leaving the laboratory, and these were kindly taken in charge by Dr. Smith for titration. In one of these he found that while the membrane was macroscopically unaltered, microscopically it contained fungi. This finding, even though it be a single instance, vitiates the value of all of those results in which an acid end reaction was found with bacilli of bovine type except one about to be mentioned. On the other hand, it may be best to consider for the moment the chance that this sort of contamination was exceptional. In that case a number of instances could be cited to show that the cultures of bovine type from human source gave a pronounced acid end reaction in the beginning which gradually became less in subsequent titrations, months later (07.8, 07.9, 07.53). Duval (5) states that his culture (I) suddenly changed after a number of months from the acid to the alkaline type. Keeping in mind this possibility of a change under cultivation, the work can never be repeated with these cultures.

In view of my experience, it may be said that five of the six non-virulent cultures have given the acid end reaction characteristic of the human type. One (07.54) has not done so. The last grows as well as the others and has from the beginning, but after months of growth, the flasks remain at about the neutral point which they gradually attain in the first few weeks. This culture, with Duval's (5) culture (IV), and a culture (M), said to have been isolated by Dr. Ravenel some years ago, are three recorded exceptions to the rule of Smith, that the human type of tubercle bacillus gives a distinctive acid end reaction on glycerine bouillon.

With six of the virulent cultures, working recently, that is, when

the cultures were over eighteen months under cultivation, a perfectly characteristic bovine curve has been obtained. In a seventh culture (07.8), proved of bovine type, even in respect to its virulence for calves, the lowest end reaction obtained has been 1.3 per cent. acid (initial acidity 1.5 per cent.). This, while a lower reaction than any of the five cultures of human type, is not characteristic. In the case of the two remaining cultures, the reaction curve could not be determined as the cultures could not be established on the medium.

The results of the reaction determinations have been generally in accord with the classification of the cultures on the basis of cultural characters and virulence. Culture 07.54 is an exception. Duval (5), in the case of his similar culture (IV), concluded that he had to deal with an attenuated bovine type. This question may well be reserved to await future results. That cultures of bovine type may, when recently isolated from the human body, present an abnormal reaction curve, is not in any way rendered improbable by the results already reported by others, most of which were obtained with cultures over a year under cultivation; but it is no more than a vague possibility raised by results otherwise unsatisfactory.

Clinical Data.—In closing, it may be noted that a careful analysis of such clinical and pathological data as I have been able to gather in connection with these cases has shown no feature of distinction between the group of cases in which the human and bovine types of bacilli were found, in respect to the lesion, number of bacilli present, duration or recurrence of the disease, family history, previous history or present condition of the patients. There is, however, a striking difference in the age of patients. The average age of eight of the patients giving bovine type of bacillus was 8½ years. The youngest was 18 months and the oldest 18 years. The average age of six patients yielding the human type of bacillus was 17⅔ years, the oldest was 32 years, and youngest 8 years. Of the cases of cervical gland tuberculosis in persons over 15 years of age, four-fifths were infections with human type. Of the cases in persons below 15 years of age, seven-ninths were of bovine type. Hence the bovine type of infection would seem to be more common in children and the human in young adults.

SUMMARY.

The examination of fifteen consecutive cases of primary tuberculous cervical adenitis resulted in the isolation of nine cultures of *B. tuberculosis* of bovine type and six cultures of human type, the classification being made on the basis of adaptability to artificial cultivation, character of growth on glycerine bouillon, virulence for rabbits, and, in three instances, virulence for calves.

The results of the determination of the reaction changes in glycerine bouillon due to the growth of the cultures on this medium were in general accord with the classification as based on other characters. One culture otherwise of human type was found to give the slightly acid or even alkaline end reaction characteristic of the bovine type of bacillus. One culture, otherwise of bovine type, has so far given an end reaction more highly acid than is usual with bovine cultures. Two cultures, highly virulent for rabbits, showed even less adaptability to artificial cultivation than the usual true bovine cultures and the reaction change could not, therefore, be determined.

BIBLIOGRAPHY.

1. Smith, T., and Brown, H. R., Studies in Mammalian Tubercle Bacilli, IV, *Jour. of Med. Research,* 1907, xi, 435.
2. Weber and Taute, Weitere Untersuchungen ueber Tuberkelbazillen. . . . *Tuberkulose Arb. a. d. k. Gsndhtsamte,* 1907, No. 6, p. 15.
3. Second Interim Report of the Royal Commission on Human and Bovine Tuberculosis, 1907, Part I.
4. Oehlecker, Untersuchungen ueber chirurgische Tuberkulosen, *Tuberkulose Arb. a. d. k. Gsndhtsamte,* 1907, No. 6, p. 88.
5. Duval, C. W., Studies in Atypical Forms of Tubercle Bacilli. . . . *Jour. of Exper. Med.,* 1909, xi, 403.
6. Park, W. H., Sixth International Congress on Tuberculosis, Washington, 1908, Vol. iv, Pt. 2, p. 697.
7. Smith, T., A Comparative Study of Bovine Tubercle Bacilli and of Human Bacilli from Sputum, *Jour. of Exper. Med.,* 1898, iii, 451.
8. Smith, T., *Trans. of the Assn. of American Physicians,* 1903, xviii, 108.
9. Beitzka, *Virchows Arch.,* 1907, cxc, Suppl., 58.
10. Second Interim Report of the Royal Commission on Human and Bovine Tuberculosis, 1907, Vol. iii, Pt. ii, 55.
11. Bang, O., *Cent. f. Bakt., Orig.,* 1906, xliii, 34.

THE INFLUENCE OF ADRENALIN IN PHLORHIZIN DIABETES.[1]

By A. I. RINGER.

(*From the Physiological Laboratory of the New York University and Bellevue Hospital Medical College.*)

In 1901, Blum[2] made the very important discovery that the suprarenals contain a substance which, when injected into the circulation of animals, produces glycosuria. This substance which he later[3] found to be identical with adrenalin, when given by stomach, produces no effect on the carbohydrate metabolism. The glycosuria appeared in animals from whose diet all carbohydrates were excluded and in one dog's urine (Experiment 19) after seventeen days of starvation, he obtained 0.4 per cent. of sugar. In a subsequent work,[4] however, Blum found that out of seven animals which had fasted for eight days, only one reacted to adrenalin. The others did not react at all, or reacted very slightly. But, if after a period of nine days of starvation, he fed the animals for several days with olive oil, administration of adrenalin subcutaneously was followed by the appearance of from 1 to 1.5 per cent. of sugar in the urine. These facts led Blum to the conclusion that the carbohydrate excreted was derived from fat.

In a recent paper, Eppinger, Falta and Rudinger[5] report a series of experiments, which according to their interpretation, confirm Blum's theory of the source of carbohydrate in adrenalin glycosuria. They starved a dog for three days; made him run in a tread-mill one hour daily during these three days; then injected 0.01 gram of adrenalin (one milligram per kilo) subcutaneously. This was followed by an excretion in the urine of 2.83 grams of sugar in a period of eight hours.

[1] Received for publication November 10, 1909.

[2] Blum, *Deutsches Arch. f. klin. Med.*, 1901, lxxi, 146.

[3] Blum, *Arch. f. d. ges. Physiol.*, 1902, xc, 617.

[4] *Loc. cit.*

[5] Eppinger, Falta and Rudinger, *Zeit. f. klin. Med.*, 1908, lxvi, 1.

To a second dog, they gave 200 grams of butter during the course of seventy-two hours. They followed this by a subcutaneous injection of 0.01 gram of adrenalin, and obtained 2.8 grams of sugar during the first period of five hours, and 3.9 grams during the second period of seven hours. The authors attribute this increase in the sugar elimination to the ingestion of the butter.

One must not, however, consider these experiments as conclusively proving the conversion of fat into carbohydrate. Blum as well as Eppinger, Falta and Rudinger take it for granted that the animals, in the experiments cited, were glycogen free after fasting for seventeen days in the dogs of the former, and after three days with one hour of daily work in those of the latter. But Pflüger[6] has shown that a dog after fasting for twenty-eight days and after losing 23 per cent. of its body weight still contains glycogen enough to give rise to 100 grams of sugar, and Prausnitz[7] reports that a dog weighing 22 kilograms after fasting for twelve days and after excreting 287 grams of sugar in the urine, brought about by phlorhizin injections, has still contained 25 grams of glycogen in its tissues. These two experiments show absolutely, that unless a more effective method—like convulsions, excessive work, or exposure to cold—is employed in addition, a long period of fasting does not suffice to free an animal of its glycogen.

In the experiments in which animals were fed with olive oil, the extra output of sugar after adrenalin administration, may be accounted for in the following manner. After a period of fasting, the animal body always exhibits a marked tenacity for its glycogen, and any fat available will be burnt in preference to it. The feeding of olive oil or butter to such an animal, may serve to spare the store of glycogen, even more than will body fat, and will cause its retention in the tissues. Administration of adrenalin after ingestion of fat may, therefore, be followed by a greater excretion of sugar in the urine.

In another series of experiments, Eppinger, Falta and Rudinger extirpated the pancreas of several dogs, starved them for two days, and determined the D:N ratio during this period. Then they

[6] Pflüger, *Arch. f. d. ges. Physiol.*, 1902, xci, 119.
[7] Prausnitz, *Zeit. f. Biol.*, 1892, xxix, 168.

administered adrenalin subcutaneously. Their results are here tabulated:

TABLE 17.

BROWN DOG, 8.4 KILOGRAMS. PANCREAS EXTIRPATED ON DECEMBER 27, 1907, AT 9 A. M.

Date.	Period in hours.	Remarks.	Total N.	Total D.	D:N.
Dec. 27	11	Dog fasting	2.11	2.75	1.38
"	12	"	5.27	13.77	2.52
Dec. 28	10	"	1.90	3.52	1.85
"	14	"	2.12	4.98	2.35
Dec. 29	2	"	0.4	0.87	2.2
"	6	0.008 gm. of adrenalin administered subcutaneously			
			2.97	15.56	5.2
"	6	Dog fasting	3.86	17.85	4.84
"	9¾	"	1.87	5.87	3.1

TABLE 18.

DOG'S WEIGHT, 13.9 KILOGRAMS. PANCREAS EXTIRPATED ON DECEMBER 31, 1907, AT 10 A. M.

Date.	Period in hours.	Remarks.	Total N.	Total D.	D:N.
Dec. 31, '07	10¾	Dog fasting		0.31	
"	11¼	"		4.53	
Jan. 1, '08	10½	"	2.87	5.42	2.99
"	13	"	3.75	6.54	1.77
Jan. 2	11½	"	3.85	5.50	1.43
"	15	"	3.44	7.65	2.23
Jan. 3	9½	"	2.24	4.65	2.07
"	4	0.012 gr. adrenalin injected subcutaneously			
			1.36	8.55	6.11
"	8⅛	Dog fasting	2.34	6.46	2.76
Jan. 4	4¾	50 gm. of butter + 5 gm. of pancreatin			
			1.484	2.814	1.9
"	5½	Dog fasting	1.61	2.69	1.6

After the adrenalin injections, they obtained a D:N ratio of 5.2 (after six hours) and 6.1 (after four hours) respectively in Tables 17 and 18. Since the usual D:N ratio in depancreatized dogs is 2.8,[8] the authors come to the conclusion that all the extra sugar is unquestionably derived from fat.

Tliis appears to be true only upon a superficial examination of the figures, but upon a closer analysis an entirely different conclusion may be drawn.

During the first two days of the first experiment cited (Table 17), the animal excreted 11.8 grams of nitrogen and 25.89 grams of sugar. The D:N ratio was 2.194. This ratio shows that the

[8] Minkowski, *Arch. f. exper. Path. and Pharm.*, 1893, xxxi, 85.

animal was not completely diabetic and that 7.15 grams of sugar[9] were either oxidized or stored as glycogen. During the twenty-four hours following the injection of adrenalin, the animal excreted 8.7 grams of nitrogen and 39.28 grams of sugar. The D:N ratio was 4.51. From this we see that there was an extra elimination of 14.92 grams of sugar.[10] This extra sugar, which was about 0.2 per cent. of the body weight, may have come from the fluids of the body; because after adrenalin injection, the blood sugar content very often sinks to subnormal (Vosburgh and Richards).[11] Or else it may have been derived from the glycogen, which had not yet been liberated from the tissues, since the diabetes was not complete.

In the second experiment (Table 18), we find that the animal excreted during the foreperiod 16.15 grams of nitrogen and 29.76 grams of sugar, with a D:N ratio of 1.84, which also signifies an incomplete diabetes, and shows that the animal either burnt or stored 15.46 grams of sugar.[12] During the twenty-three hours following the adrenalin injection the animal excreted 6.79 grams of nitrogen and 20.51 grams of sugar. The D:N ratio was 3.02, (not 6.11 as in the first four hours of this period)—a not unusual ratio for pancreas diabetes (Minkowski).[13]

In the foregoing, we have seen that the most important arguments in favor of the theory that adrenalin converts fat into carbohydrates may have been based upon false premises from the beginning. No experiments in Professor Lusk's[14] laboratory have ever furnished the slightest reason to believe that sugar can be produced from the metabolism of body fat. To investigate further the correctness of this view, several experiments were performed in accordance with the following principle.

Professor Lusk[15] has shown that if dogs be placed completely under the influence of phlorhizin, diabetes results. The glycosuria bears a constant relationship to the nitrogen, *i. e.,* for every gram

[9] $(11.8 \times 2.8) - 25.89 = 7.15$.
[10] $39.28 - (8.7 \times 2.8) = 14.92$.
[11] Vosburgh and Richards, *American Jour. of Physiol.,* 1903, ix, 35.
[12] $(16.15 \times 2.8) - 29.76 = 15.46$.
[13] *Loc. cit.*
[14] Lusk, *American Jour. of Physiol.,* 1908, xxii, 163.
[15] Stiles and Lusk, *American Jour. of Physiol.,* 1903, x, 67.

of nitrogen excreted in the urine, we find approximately 3.65 grams of sugar. If we take an animal like this, and by exposing it to cold render it glycogen free,[16] any intraperitoneal injection of adrenalin, ought to be followed by a rise in the D:N ratio, provided the theory of a conversion of fat into carbohydrate is true. That this is not the case will be seen from the accompanying protocols.

Methods.

The adrenalin used was that prepared by Parke, Davis & Co. In six of the seven dogs experimented on, the dose given was the same as that employed by Eppinger, Falta and Rudinger, *i. e.*, one milligram per kilogram of body weight. But this dose proved fatal within twenty-four hours in all cases except in Dog V. So in Dog VII the dose was reduced to 0.5 milligrams per kilogram.

The phlorhizin used was that prepared by Merck. It was administered subcutaneously in two gram doses three times a day, dissolved in warm 1.2 per cent. sodium carbonate solution.

The total nitrogen was determined by Kjeldahl method and the total sugar by Allihn's gravimetric method.

Dog No. 5.

March 6, '09.	Dog fasting.
March 7, '09.	Dog fasting.
March 8, '09.	2 gm. of phlorhizin injected at 8 A. M., 3.30 P. M., 10 P. M.
March 9, '09.	Dog fasting. At 5:15 P. M. the dog was given a bath at a temperature of 8° C. for 30 minutes and while wet was placed in a cold room for 5½ hours.
March 10, '09, 9:30 A .M.	2 gm. phlorhizin injected subcutaneously.
March 10, '09, 10:15 A .M.	Catheterized and bladder washed.

TABLE I.

Time.	No. of hours.	Weight of dog.	Total N.	N per hour.	Total D.	D per hour.	D:N.
10:15 A. M.–1:15 P. M.	3	9.12	1.57	0.523	5.168	1.723	3.29
1:15 P. M.	0.009 gm. of adrenalin injected intraperitoneally						
1:15 P. M.–8:45 A. M.[17]	19½		8.072	0.414	25.928	1.33	3.21

[16] Lusk: *Loc. cit.*

[17] At 4:10 P. M. and 10:45 P. M., 2 gm. of phlorhizin were injected subcutaneously.

This animal was kept in a cold room for five and a half hours following a period of four days of starvation, inclusive of two days of phlorhizin diabetes. This has apparently rendered the animal glycogen free. Administration of adrenalin, therefore, produced no change in the D : N ratio.

Dog No. 7.

April 10, '09. Dog fed last.
April 11, '09. Dog starving.
April 12, '09.
April 13, '09. Dog starving. 2 gm. of phlorhizin at 8:50 A. M., at 4 P. M. and at 11 P. M.
April 14, '09. Dog starving. At 5 P. M. dog given a cold bath at temperature of 5° C. for 30 minutes. Then placed in cold room for the night.
April 15, '09, 8:50 A. M. 2 gm. of phlorhizin injected subcutaneously.
April 15, '09, 10:25 A. M. Catheterized and bladder washed. Dog's weight 8.76 kg.

TABLE II.

Time.	No. of hours.	Weight.	Period.	Condition.	Total N.	N per hour.	Total sugar.	Sugar per hour.	D : N.	Remarks.
10:25 A. M.–3:25 P. M.	5:00	8.76	I.	Phlorhizin	2.264	0.453	7.248	1.4496	3.2	
3:25 P. M.–8:25 A. M.	17:00		II.	Adrenalin and phlorhizin	6.297	0.37	30.176	1.775	4.79	(at 3:25, injected 0.005 gr. adrenalin intraperitoneally)
April 16. 8:25 A. M.–3:40 P. M.	7:15		III.	Phlorhizin	2.973	0.41	9.376	1.29	3.12	
3:40 P. M.–8:40 A. M.	17:00		IV.	Adrenalin and phlorhizin	7.129	0.419	23.92	1.407	3.35	(at 3:40, .005 gr. adrenalin injected)
April 17. 8:40 A. M.–3:40 P. M.	7:00		V.	Phlorhizin	2.973	0.426	9.264	1.323	3.116	
3:40 P. M.–8:40 A. M.	17:00		VI.	Adrenalin and phlorhizin	6.687	0.393	21.824	1.284	3.26	(at 3:40, .005 gr. adrenalin injected)

Throughout the course of the experiment the phlorhizin was injected regularly at 8:00, 3:45 and 11:55.

April 19, death of dog.

This animal was treated in a similar manner to Dog No. 5, but, because the April night was not as cold as the night of March, this dog was not sufficiently chilled to exhaust it of all its glycogen. The first administration of adrenalin was, therefore, followed by a rise in the D:N ratio, showing that a sweeping out or "Ausschwemmung" of the carbohydrates of the tissues took place. The second and third administration of adrenalin, however, failed to produce any extra sugar elimination, because the first one had, undoubtedly, removed all the glycogen that was present.

THE MECHANISM OF ADRENALIN GLYCOSURIA.

A number of theories have been advanced by the different investigators with regard to the mechanism of adrenalin glycosuria. That it is not of renal origin, like phlorhizin glycosuria has been shown by Zuelzer,[18] and this has been corroberated by Metzger.[19] Both authors obtained a decided hyperglycemia on injecting adrenalin after nephrectomy. Vosburgh and Richards[20] found that the hyperglycemia is more intense in well-fed animals than in starved ones. They also found that the blood of the hepatic vein contains a higher percentage of reducing substances than the portal vein, suggesting very strongly that the liver is responsible for the hyperglycemia. Herter[21] believes that adrenalin exerts a toxic effect on the cells of the pancreas, by interfering with its function in some unknown way. Eppinger, Falta and Rudinger[22] attribute to adrenalin a series of functions. They believe that it has the power of mobilizing the carbohydrates, and, at the same time, of inhibiting the production of the internal secretion of the pancreas. The glycosuria, therefore, results from the lack of a corresponding increase in the pancreatic ferments. Underhill and Closson[23] think that the adrenalin stimulates the nervous system, probably the sym-

[18] Zuelzer, *Berliner klin. Woch.*, 1901, xlviii, 1209.

[19] Metzger, *Münch. med. Woch.*, 1902, xii, 478.

[20] *Loc. cit.*

[21] Herter, *Medical News*, 1902, lxxx, 865; lxxxi, 769; Herter and Wakeman, *Virchow's Arch.*, 1902, clxix, 479; Herter and Richards, *Medical News*, 1902, lxxx, 201.

[22] *Loc. cit.*

[23] Underhill and Closson, *American Jour. of Physiol.*, 1906, xvii, 42.

pathetic. This stimulation causes the liver and the other glycogen storehouses to throw out their glycogen in the form of dextrose. More dextrose than can be cared for appears suddenly in the blood and glycosuria is the result.

Opposed to the above theories are the results obtained by Straub[24] and Ritzmann.[25] They found that a continuous infusion of an adrenalin solution with a concentration of 1:2,000,000, flowing at a rate of two cubic centimeters per minute, i. e., an increase in 0.000001 gram of adrenalin secretion into the blood per minute, may go on indefinitely without producing any glycosuria. Kretschmer[26] found this amount and rate of flow to be below the minimum that is required to bring about a rise in blood pressure. On the other hand, the infusion of the same adrenalin solution at a rate of four cubic centimeters per minute, which according to Kretschmer produces a considerable rise in blood pressure, results in an almost immediate appearance of sugar in the urine. Straub and Ritzman also found a constant relationship existing among the amount of adrenalin injected into the blood and the degree of increased blood pressure and the amount of sugar in the urine.

This suggests very strongly that the glycosuria following adrenalin injections is the result of an anemia produced by the constriction of the blood vessels. The anemic condition of the tissues results in imperfect oxidation, and whenever this condition occurs there is a demand on the glycogen reserve of the body, so that hyperglycemia may arise, and both sugar and lactic acid appear in the urine (Zuntz).[27] This hyperglycemia and glycosuria, which is similar to the hyperglycemia and glycosuria obtained in asphyxiation (Macleod)[28] outlasts the stimulus.

The writer's thanks are due to Professor Lusk, at whose suggestion and under whose direction this investigation was conducted.

[24] Straub, *Münch. med. Woch.*, 1909, lvi, 493.
[25] Ritzmann, *Arch. f. exper. Path. und Pharm.*, 1909, lxi, 231.
[26] Kretschmer, *Arch. f. exper. Path. und Pharm*, 1907, lvii, 423.
[27] Zuntz, Festschrift, Die Kraftleistung des Tierkörpers, Berlin, 1908, p. 18.
[28] Macleod, *American Jour. of Physiol.*, 1909, xxiii, 278.

CONCLUSIONS.

I. Intraperitoneal injections of adrenalin into animals which are completely under the influence of phlorhizin and which are free from glycogen do not result in any extra elimination of sugar. This proves that adrenalin does not cause a conversion of fat into carbohydrates, as is maintained by Blum and by Eppinger, Falta and Rudinger.

II. The high D:N ratio 5.2 and 6.1 reported by Eppinger, Falta and Rudinger after adrenalin injections in depancreatized dogs, which were obtained after short periods of six hours and four hours respectively, can be explained by the elimination of carbohydrates present in the organism.

III. These results confirm the work of Straub and Ritzmann which indicates that adrenalin, by its constricting effect on the blood vessels, produces anemia of the tissues, resulting in imperfect oxidation, and this anemia is followed by the conversion of glycogen into dextrose by hyperglycemia and consequently by glycosuria.

THE FEW COMPLETE SETS OF THE JOURNAL OF EXPERIMENTAL MEDICINE, VOLS. I TO VI INCLUSIVE, remaining are now offered for sale, bound in cloth, at the price of $40.00 net per set. (Including Vols. VII, VIII, IX, X and XI, unbound, the price will be $65.00.) (Vol. I contains 716 pages and 75 illustrations ; Vol. II, 735 pages and 67 illustrations; Vol. III, 684 pages and 75 illustrations; Vol. IV, 654 pages and 47 illustrations; Vol. V, 657 pages and 56 illustrations; Vol. VI, 552 pages and 47 illustrations.)

The price of the sets may be advanced at any time. Orders may be addressed to

THE JOHNS HOPKINS PRESS, BALTIMORE, MD.

or to

THE JOURNAL OF EXPERIMENTAL MEDICINE, 66th STREET AND AVENUE A, NEW YORK

CONTENTS.

WINTERNITZ, M. C., and PRATT, J. P. On the Relation of the Catalytic Activity of the Blood to the Number of Red Blood Cells in Health, and to the Number of White Blood Cells and the Body Temperature in Peritonitis 1

WINTERNITZ, M. C., and ROGERS, W. B. The Catalytic Activity of the Developing Hen's Egg 12

DARLING, SAMUEL T. Experimental Sarcosporidiosis in the Guinea-pig and its Relation to a Case of Sarcosporidiosis in Man. PLATES I and II 19

WOGLOM, WILLIAM H. Resistance Produced in Mice against Transplanted Cancer by Auto-inoculation of the Spleen . 29

MELTZER, S. J., and AUER, JOHN. The Respiratory Changes of Pressure at the Various Levels of the Posterior Mediastinum. PLATE III 34

COOKE, JEAN V. The Excretion of Calcium and Magnesium after Parathyroidectomy 45

STEWART, HUGH A. The Dextrose Consumption by the Isolated Perfused Human Heart 59

MEAKINS, J. C. An Experimental Study of Opsonic Immunity to Staphylococcus Aureus 67

LEWIS, PAUL A. Tuberculous Cervical Adenitis: A Study of the Tubercle Bacillus Cultivated from Fifteen Consecutive Cases 82

RINGER, A. I. The Influence of Adrenalin in Phlorhizin Diabetes 105

The Journal of Experimental Medicine is issued bimonthly. A volume contains at least six hundred pages, many plates and figures.

Papers for publication may be sent to the editors at the Rockefeller Institute for Medical Research, 66th Street and Avenue A, New York. Other communications should be addressed to *The Journal of Experimental Medicine* in care of the Rockefeller Institute for Medical Research.

Contributors are requested to limit the length of articles submitted to the Journal of Experimental Medicine to twenty printed pages. Articles which exceed in length twenty-five printed pages will be returned to the authors in order that their contents may be reduced to this maximum, which does not include the space occupied by illustrations.

Authors receive fifty copies of their papers free; additional copies may be obtained at cost.

The subscription price is $5.00 (21 shillings, 21 marks, 26 francs) per volume. Subscriptions may be sent directly to *The Journal of Experimental Medicine*, 41 North Queen Street, Lancaster, Pa., or 66th Street and Avenue A, New York; or to The Macmillan Company, London; Mr. Gustav Fock, Leipzig; and Messrs. Masson et Cie, Paris.